THE INFLUENCE OF PHYSICAL ACTIVITY ON CHARACTERISTICS OF THE FIBRIN NETWORK STRUCTURE OF ACTIVE AND SEDENTARY MALES

Sarah Johanna Moss
B.Sc., Hons B.Sc (Biochemistry), Hons B.Sc. (Biokinetics), M.Sc. (Biochemistry)

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Promoter: Dr L.I. Dreyer
Co-Promoter: Prof. H.H. Vorster

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Jesus looked at them and said, "With man this is impossible, but not with God; all things are possible with God."

Mark 10:27

Fibrin network structure with red cells
Incredible Voyage : exploring the human body
(National Geographic Society, 1998)

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ABSTRACT

Background

In 1990 cardiovascular disease (CVD) was the main cause of death world wide (Murray & Lopez, 1996). Regular physical activity is associated with a lower risk of CVD. The mechanisms through which regular physical activity reduces the risk of CVD is not fully understood. The independent, probably causal association of increased plasma fibrinogen with CVD is established (Meade *et al.*, 1986). This association seems to be stronger than that of cholesterol with CVD (Ernst *et al.*, 1992). It is hypothesised that part of this association may be mediated by the quality of fibrin network structures formed when fibrin(ogen) monomers polymerise. The plasma metabolic environment determines the characteristics of the fibrin network structure that is formed. Regular physical activity results in changes in the metabolic environment, which is expected to influence formation of fibrin network structures. Changes in diet also lead to changes in the metabolic environment. When a diet consisting of a high glycaemic index (GI) is ingested, large variations in blood glucose and insulin responses have been found, while the ingestion of a low GI meal results in the slow release of glucose and insulin with less variations (Wolever, 1990). The effect of physical activity and a combination of activity with a low or high GI pre-exercise meal on the formation of the fibrin network structures are not known.

Objectives

The purpose of this study was to determine the influence of a maximal exercise bout on fibrin network characteristics. The combinations of a low or high GI pre-exercise meal and physical activity on the network structure characteristics were also investigated.

Methods

Fifteen active and 14 sedentary males were recruited to participate voluntarily in the study. The respondents were subjected to a maximal exercise, blood samples taken fasting, during maximal activity, and after 30 minutes of recovery. Maximal activity was achieved by exercising the respondents to exhaustion on a Monark bicycle ergometer. The resistance was increased with 50 watt increments after every four minutes. Maximal

activity was reached when the heart rate reached aged predicted maximum (according to Karvonen's equation), or the respondent could not continue. The heart rate and blood pressure was recorded after 3 minutes in every stage before the resistance was increased.

The characteristics of the fibrin network structure determined were the permeability coefficient (K_s), mass-length ratio (MLR) and compaction. These were interpreted against the background of changes in several haemostatic variables in plasma. To determine the influence of the GI of a meal, the same study design was used with a high and low pre-exercise GI meal given in a random order. Blood samples were taken fasting and at 1-hour post meal, maximal exercise, and after 30 minutes of recovery. The K_s, MLR, compaction and selected biochemical variables were determined at all the times.

Results

Maximal exercise resulted in an increase in K_s of the fibrin network structure from plasma of active males, while the MLR decreased when compared to that at rest. After 30 minutes of recovery the K_s decreased slightly, but remained higher than the K_s obtained at rest. The MLR increased after 30 minutes of recovery to values significantly higher than at rest. It seems that the active males formed fibrin network structures that were less resistant to lysis (Blombäck *et al.*, 1990) after 30 minutes of recovery than at the start of activity. This is possibly due to the fibrin network structure being more permeable with shorter and thicker fibres. In the sedentary males, the K_s decreased while the MLR increased in response to the maximal activity. After 30 minutes of recovery the K_s of the sedentary males decreased even more, to result in less permeable fibrin network structures than before activity. The MLR increased with maximal activity, but after 30 minutes of recovery decreased to values lower than before activity. It seems that the sedentary males formed fibrin network structures after maximal activity that were less permeable and more resistant to lysis, possibly due to longer and thinner fibres.

After ingestion of the GI meal, the network structures were characterised 1 hour post-meal, at maximal activity and after 30 min of recovery. The low GI meal resulted in a smaller insulin response together with an increase in compaction in comparison with the high GI meal. In the case of the sedentary group the low GI meal also had a smaller insulin response, with an increase in MLR. This increased MLR suggests that shorter and thicker

fibrin network structures are formed with the ingestion of a low GI meal when compared to a high GI meal.

The advantage of the low GI meal compared to the high GI meal was seen in the increased MLR and compaction in response to maximal activity in the active males. A slight increase was also seen in K_s. This suggests that the active males formed more permeable fibrin network structures that are more readily dissolved than those formed with the high GI meal. The low GI meal also increased compaction and less fluctuation in insulin levels were found in the active males. The results found in the sedentary males were not clear. The characteristics of the fibrin networks indicated a trend towards a decrease in K_s while the high GI meal decreased compaction and the low GI meal increased compaction.

Recovery from the maximal exercise resulted in a decrease in K_s of the active males with both the high and low GI meals. The low GI meal resulted in values higher than initially measured at fasting. The MLR showed similar changes. MLR values were higher after activity in combination with the low GI meal than with the high GI meal. The same trend was found in the sedentary males, but the MLR and K_s values after recovery in combination with the high GI meal were lower than at fasting, values returned to the fasting values when the low GI meal was eaten. An increase in K_s suggests an increase in permeability of the fibrin network structure, while an increase in MLR, shorter and thicker fibrin fibres. (Blombäck *et al.*, 1990). These types of networks are less thrombogenic and less resistant to lysis. A decrease in these characteristics will have the opposite effect with more lysis-resistant fibrin network structures being formed.

Conclusion

It is concluded that the effects of physical fitness and acute bouts of intensive exercise on fibrin networks formed from plasma of healthy young males may differ. These fibrin networks formed from plasma of healthy young males are influenced by the glycaemic properties of the pre-exercise meal. Changes in the metabolic environment occurred that possibly affected the characteristics of the fibrin network structures during exercise and after meals. More research is needed for a better understanding of underlying mechanisms, and to relate these differences to health outcomes.

Key words

physical activity, males, fibrin network structure, glycaemic index, pre-exercise meal

Congress presentations

Findings of this study have been presented at the following conferences:

- Moss, S.J., Smith, E., Malan, M.M., Dreyer, L.I., & Vorster, H.H. The effect of maximal exercise on the fibrin network structure of males. The Biennial Congress of the South African Alliance for Sport Science, Physical Education and Recreation, August 1998, Durban, South Africa.

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Publications

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OPSOMMING

Agtergrond

Kardiovaskulêre siektes (KVS) was in 1990 wêreldwyd die hoofoorsaak van sterftes (Murray & Lopez, 1996). Gereëlde fisieke aktiwiteit lei tot 'n verlaging van die meeste risikofaktore wat aanleiding gee tot KVS. Die meganismes waardeur gereëlde fisieke aktiwiteit die risiko vir KVS verminder, is egter nie volledig bekend nie. Fibrinogeen is as 'n onafhanklike, moontlik oorsaaklike risikofaktor vir KVS (Meade *et al.*, 1986). Die verband is selfs sterker as dié met cholesterol (Ernst *et al.*, 1992). Die hipotese is gestel dat die assosiasie van fibrinogeen met KVS moontlik deur die kwaliteit van die gevormde fibriennetwerk-struktuur, wanneer fibrinogeen-monomere polimeriseer, bepaal word. Die interne omgewing bepaal die eienskappe van die fibriennetwerk-struktuur wat gevorm word. Fisieke aktiwiteit gee aanleiding tot veranderinge in die interne omgewing en daar word verwag dat die eienskappe van die fibriennetwerk-struktuur daardeur beïnvloed sal word. Veranderinge in dieet gee ook aanleiding tot veranderinge in die interne omgewing. Die inname van 'n hoë glukemiese indeks (GI) maaltyd lei tot 'n groot skommeling in glukose en insulien terwyl 'n lae GI maaltyd 'n stadige vrystelling van glukose en insulien tot gevolg het, met kleiner skommelinge (Wolever, 1990). Die effek van fisieke aktiwiteit, asook 'n kombinasie van fisieke aktiwiteit en 'n hoë en lae GI maaltyd op die eienskappe van die fibriennetwerk-struktuur is egter onbekend.

Doelstelling

Die doel van hierdie studie was om die effek van 'n maksimale oefensessie op die eienskappe van die fibriennetwerk-struktuur te bepaal. Hiermee saam is die effek van fisieke aktiwiteit in kombinasie met 'n lae en hoë GI maaltyd ook ondersoek.

Metodes

Vyftien aktiewe en 14 sedentêre manlike proefpersone is genader om vrywillig aan die studie deelteneem. Die mans is aan 'n maksimale oefensessie onderwerp, waartydens bloedmonsters vastend, direk na inspanning en 30 minute na herstel geneem is. Die maksimale inspanning is deur middel van 50 watt inkrimente op 'n Monark fiets ergometer uitgevoer. Die weerstand is na elke 4 minute verhoog totdat die makismale

harttempo (volgens Karvonen se vergeyking) bereik is, of die proefpersone maksimale uitputting ervaar het. Die harttempo en bloeddruk is 3 minute na elke weerstandsverandering gemeet, voordat die weerstand verhoog is vir die volgende vlak.

Die eienskappe van die fibriennetwerk-struktuur is deur middel van die deurlaatbaarheidskoëffisiënt (K_s), massa-lengte verhouding (MLR) en kompaksie, tesame met 'n paar hemostatiese veranderlikes, bepaal. Dieselfde studieontwerp is gebruik om ewekansig 'n lae en hoë GI-maaltyd se effek voor, tydens en na maksimale aktiwiteit te meet. Bloed is vastend, 1 uur na die maaltyd, na maksimale inspanning en na 30 minute herstel getrek. Die K_s, MLR, kompaksie en selektiewe biochemiese veranderlikes is oor die verskillende tye bepaal.

Resultate

Die resultate van die studie het getoon dat 'n maksimale oefensessie 'n toename in K_s en 'n afname in die MLR van die fibriennetwerk-struktuur eienskappe van aktiewe mans het wanneer dit met die rustende waardes vergelyk word. Na 30 minute herstel het die K_s effens gedaal, maar steeds hoër waardes getoon as rustend. Die MLR het toegeneem na 30 minute van herstel tot betekenisvol hoër waardes as rustend. Dit is 'n aanduiding dat die aktiewe mans fibriennetwerk-strukture gevorm het wat minder weerstand bied teen afbraak (Blombäck *et al.*, 1990) as voor die aanvang van 'n maksimale oefensessie. Dit word toegeskryf aan 'n fibriennetwerk-struktuur wat meer deurlaatbaar met korter en dikker vesels is. In die sedentêre groep het die K_s afgeneem terwyl die MLR toegeneem het in reaksie op die maksimale inspanning. Na 30 minute herstel het die K_s van die sedentêre mans verder afgeneem om sodoende minder deurlaatbare netwerke te vorm as by aanvang. Die MLR het toegeneem met maksimale inspanning, maar het na 30 minute herstel gedaal na laer waardes as tydens rustend. Die gevolg was dat die sedentêre mans fibriennetwerk-strukture na maksimale aktiwiteit gevorm het wat minder deurlaatbaar was en wat meer weerstand bied teen afbraak as gevolg van langer en dunner vesels wat gevorm is.

In die aktiewe proefpersone het die lae GI maaltyd 'n kleiner insulin respons tot gevolg gehad en 'n toename in kompaksie in vergelyking met die hoë GI maaltyd. In die geval van die sedentêre groep is ook gevind dat die lae GI maaltyd kleiner insulien response uitgelok het, tesame met 'n toename in MLR. Hierdie toename in MLR beteken dat korter

en dikker fibriennetwerk-strukture gevorm het wanneer dit in vergelyking met die hoë GI-maaltyd ingeneem is.

In reaksie op maksimale inspanning toon die toename in MLR en kompaksie die voordeel van die lae GI maaltyd in vergelyking met 'n hoë GI maaltyd by aktiewe persone aan. Die K_s het ook effens toegeneem. Dit beteken dat die aktiewe persone tydens 'n maksimale inspanning meer deurlaatbare netwerke wat maklik oplos gevorm het in vergelyking met die hoë GI maaltyd. Die lae GI maaltyd het dus kompaksies verhoog en insulien minder laat fluktueer by die aktiewe persone. Die resultate verkry van die sedentêre persone is moeiliker om te interpreteer. Die fibriennetwerk-struktuur se gemete eienskappe toon met beide maaltye 'n afname in K_s terwyl die hoë GI maaltyd kompaksie laat afneem en 'n lae GI maaltyd kompaksie laat toeneem het. Geen duidelike veranderinge is by die sedentêre proefpersone aangetoon nie en verdere navorsing is nodig.

Herstel na die oefensessie het gelei tot 'n afname in K_s by die netwerke van die aktiewe persone met beide GI maaltye, alhoewel dit hoër geëindig het na die lae GI maaltyd as by aanvang. Die MLR het dieselfde tendens getoon, met waardes betekenisvol hoër na aktiwiteit in kombinasie met 'n lae GI maaltyd as 'n hoë GI maaltyd. In die sendentêre persone is dieselfde tendens gevind, maar die MLR en K_s waardes in kombinasie met die hoë GI maaltyd het laer geëindig as met aanvang terwyl die lae GI maaltyd se herstelwaardes vir MLR en K_s na die aanvangswaardes terug gekeer het. 'n Toename in K_s beteken 'n verhoging in die deurlaatbaarheid van die netwerke, terwyl 'n toename in MLR korter en dikker netwerke beskryf (Blombäck *et al.*, 1990). Hierdie netwerke is minder trombogenies en word makliker afgebreek. 'n Afname in hierdie karakteristieke het die omgekeerde tot gevolg en resulteer in netwerke wat meer weerstand bied teen afbraak.

Gevolgtrekking

Die gevolgtrekking wat uit hierdie studie gemaak kan word, is dat die effek van inoefening en akute oefensessies op die fibriennetwerk-struktuur verskil. Die fibriennetwerk-struktuur word verder ook beïnvloed deur die GI-indeks van 'n voor-oefening maaltyd. Daar vind veranderinge in die metaboliese omgewing plaas wat 'n effek op die fibriennetwerk karakteristieke het. Verdere navorsing is nodig om onderliggende meganismes en moontlike invloed op gesondheid te verstaan.

Sleutelterme

fisieke aktiwiteit, mans, fibriennetwerk-strukture, glukemiese-indeks, voor-oefening maaltyd

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LIST OF ABBREVIATIONS

A	
ACSM	American College of Sports Medicine
ADP	Adenosine diphosphate
ALP	Alkaline phosphatase
ALT	Alanine animotransferase
APC	Activated protein C
APTT	Activated partial thromboplastin time
ARIC	Atherosclerosis Risk In Communities Study
AST	Aspartate aminotransferase
AT	Anti-thrombin
AT III	Antithrombin III
B	
BF	Body fat
BMI	Body-mass index
BP	Blood pressure
Bpm	Beats per minute
C	
CAD	Coronary artery disease
CENQAM	Centre for the Quality Assurance of Medicines
CHD	Coronary heart disease
CHS	Cardiovascular Health Study
CHO	Carbohydrates
CSCS	Caerphilly and Speedwell Collaborative Study
CV	Coefficient of variation
CVD	Cardiovascular disease
D	
D-dimer	D-dimer
DNA	Deoxyribonucleic acid
E	
EAS	Edinburgh Artery Study
EDTA	Ethylenediaminetetra-actetic acid

e.g.	exempli gratia (for example)
E-PFP	Essentially platelet free plasms
F	
FAO	Food and Agriculture Organization of the United Nations
Fib	Fibrinogen
FbDP	Fibrin degradation products
FgDP	Fibrinogen degradation products
FM	Fibrin monomer
FPA	Fibrinopeptide A
FPB	Fibrinopeptide B
FVII	Factor VII
G	
GGT	Gamma glutamyl transferase
GI	Glycaemic index
GRIPS	Goettingen Risk, Incidence and Prevelance Study
GS	Gottenborg Study
H	
HDL	High density lipoprotein
HR	Hazard ratio
HSF	Hepatocyte stimulating factor
I	
IAT	Individual anaerobic threshold
IHD	Ischaemic heart disease
IL-6	Interleukin-6
IU	International units
K	
K_s	Permeability coefficient
kJ	kiloJoule (Energy intake)
K_3EDTA	Potassium-ethylenediaminetetra-aceticacid
L	
LD	Lactate dehydrogenase
LDL	Low density lipoprotein
LIF	Leukemia inhibitory factor
Lp(a)	apolipoprotein A

Lp(b)	apolipoprotein B
LPS	Lipopolysaccharides
LS	Leigh Study
M	
MI	Myocardial infarction
MHR	Maximal age-adapted heart rate
MLR	Mass-length ratio
N	
Nm	Nanometer
NPHS	Northwick Park Heart Study
NRG	Nutrition research group
NS	Non-significant
O	
OR	Odds ratio
OSM	Oncostatin M
P	
PAI-1	Plasminogen activator inhibitor 1
PAI-2	Plasminogen activator inhibitor 2
PAP	Plasmin-antiplasmin complex
PFP	Platelet-free plasma
PTF 1+2	Prothrombin fragment 1+2
PPP	Platelet poor plasma
PU for CHE	Potchefstroom University for Christian Higher Education
PVD	Peripheral vascular disease
PWC_{max}	Physical work capacity at maximal heart rate
R	
RPE	Rate of perceived exertion
Rpm	Revolutions per minute
RR	Relative risk
S	
SD	Standard deviation
SHEEP	Stockholm Heart Epidemiology Program Study
SHHS	Scottish Health Heart Study
SKF	Skinfolds

SMC	Smooth muscle cells
SRR	Standardised relative risk
T	
TAT	Thrombin-antithrombin complex
TC	Total cholesterol
TFPI	Tissue factor pathway inhibitor
TG	Triglycerides
THUSA	Transition and Health during Urbanisation of South Africa study
TIA	Transient ischaemic attack
t-PA	Tissue type plasminogen activator
U	
u-PA	urokinase type plasminogen activator
V	
VO_2max	Maximal oxygen uptake
W	
W	watt (resistance)
WHO	The World Health Oganisation
Symbols	
μ_T	Mass-length ratio from turbidity
α	alpha
β	beta
γ	gamma
ϕ	fibrinogen
λ	lambda (wavelength)

CHAPTER 1

INTRODUCTION

1.1 Introduction

Coronary heart disease (CHD) was in 1990 worldwide the main cause of mortality (Murray & Lopez, 1996). These authors projected that CHD disease will still be one of the main causes of death in developed and developing countries by the year 2020. Besides the personal loss, this has an impact on health services and thus on the local economy. It is estimated that during 1991 CHD and related circulatory diseases have cost South Africa between 4.1 and 5 billion rands. This excludes rehabilitation and follow-up visits (Pestana *et al.*, 1996). A comprehensive number of studies have identified characteristics that can identify persons with a high risk for CHD. These risk factors include the smoking habit, hypertension, blood cholesterol, a high fat diet, obesity, a lack of exercise and elevated plasma fibrinogen levels (European Atherosclerosis Society, 1993). By decreasing the CHD risk factors, atherosclerosis and thrombogenesis, the incidence of myocardial infarctions and strokes will be reduced. It is estimated that in 90% of heart attacks, a blood clot superimposed on an underlying atherosclerotic plaque in the coronary artery, is the cause (Gordon & Gibbons, 1991). Recent studies have shown that elevated plasma fibrinogen is a major, independent, and one of the most important risk factors for CHD (Tanaka & Sueishi, 1993).

1.2 Problem statement

The advantages of regular physical activity on the lowering of many risk factors for CHD have been studied extensively. Paffenbarger *et al.* (1984) have found that high levels of regular physical activity constantly showed decreased levels of some CHD risk factors in

men. However, studies on the influence of physical activity on total cholesterol have indicated that independent of diet and the loss of body mass, physical activity does not have a dramatic effect on total cholesterol levels (Haskell, 1984; Tran *et al.*, 1983). Barlow *et al.* (1990) found that physical activity could lower the risk for CHD even in the presence of primary risk factors namely smoking, hypertension and an elevated total cholesterol concentration. The mechanism by which this decline is established has not yet been determined. According to Connelly *et al.* (1992) one possibility might be through the effect of physical activity on blood clotting and the haemostatic balance.

Studies directed at the effect of physical activity on blood clotting indicate an increase in tissue plasminogen activator (t-PA) (Suzuki *et al.*, 1992). The increased t-PA has the same effect on the haemostatic balance as that of a standard aspirin taken daily to prevent myocardial infarction (Gordon & Gibbons, 1991). The hypothesis of Connelly *et al.* (1992) is that the risk for CHD is lowered by physical activity, due to beneficial effects on the haemostatic balance. Boman *et al.* (1994) also concluded from their studies that an increase in fibrinolysis is orchestrated by physical activity. Studies on the influence of physical activity on plasma fibrinogen levels have resulted in contradicting results due to different protocols being used (El-Sayed, 1996a). Studies that corrected for plasma volume during acute physical activity have found no significant decrease in plasma fibrinogen levels (De Scalzi *et al.*, 1987). Significant decreases in plasma fibrinogen levels have been found in studies performed after long-term physical activity (Ernst *et al.*, 1985). Studies on the influence of physical activity on the fibrin network could, however, not be found. A direct relation between fibrinogen and the fibrin network exists, thus making it possible for physical activity to have an influence on the fibrin network structure.

The fibrin network structure forms the "hinge" of the haemostatic balance. The initiation of coagulation results in fibrinogen forming fibrin monomers. These monomers polymerise to form fibrin threads leading to the formation of the fibrin network structure. The fibrin network structure consists of a lattice-work of fibrin threads. This lattice-work forms the basis in which red blood cells and platelets are trapped, resulting in the formation of blood clots (Blombäck, 1996). These blood clots are known as thrombi when they become dislodged. Fibrin network structures with either long and thin threads, or short and wide threads are formed (Blombäck & Okada, 1983). The networks are divided into a major network composed of thicker fibres and a minor network occupying the

interstitial spaces between major network fibres (Shah *et al.*, 1982). Networks formed with long, thin threads are more resistant to lysis than networks consisting of short and thick threads (Gabriel *et al.*, 1992). Once the fibrin network structure starts forming, the fybrinolysis system is activated to dissolve the blood clots that have been formed.

Rauramaa (1986) implicated fourteen years previously that more research is needed to understand the mechanism by which physical activity modifies the development of thrombosis and thereby lowering the risk of developing CHD. This mechanism has, as yet, not been clarified. The purpose of this study was to investigate a putative mechanism by which physical activity leads to a decrease in the risk of CHD through effects on the fibrin network structure. Furthermore, the influence of a high and a low glycaemic index (GI) meal on the fibrin network characteristics was investigated. Meals with a low GI tend to cause a slower increase in post-prandial blood glucose levels, thus diminishing the insulin response and maintaining a more stable blood glucose level. On the other hand, a meal with a high GI leads to a larger fluctuation in blood glucose and insulin levels, which may influence the haemostatic balance negatively. The question to be answered with this study was what are the effects of physical activity on the characteristics of the fibrin network structure? The investigation was done in two phases. In the first phase, a comparison between the fibrin network structure of sedentary and highly active male subjects before and after an acute exercise session was made. In the second phase of the study, the influence of a high and low GI pre-exercise meal on the fibrin network characteristics during a maximal bout of activity was examined.

The value of this study is that results regarding the mechanism of how CHD risk is lowered by exercise through effects on the haemostatic balance may lead to a better understanding of the role of physical activity on CHD prevention. The implications are that by lowering thrombus formation, myocardial infarction and stroke will be reduced. This will contribute towards a healthy and more productive community, with an increased quality of life.

1.3 Hypothesis

Research has shown that plasma fibrinogen levels change during and after physical activity. Contradicting results have been obtained for plasma fibrinogen levels during acute exercise (El-Sayed, 1996a). Long term physical activity is associated with lower plasma fibrinogen levels (Ernst *et al.*, 1985). The hypothesis being tested in this study is that because physical activity will change plasma fibrinogen levels as well as the metabolic "environment" of plasma, changes in the fibrin network structure can be expected. It is hypothesised that exercise will change fibrin network structures from tighter, less porous networks with thin fibres and a high density of nodes, to more porous networks with thick fibres and fewer nodes. This will result in a structure that is easily dissolved during fibrinolysis. By comparing the network of highly active with sedentary males, information on long-term effects of physical activity will be collected. Assessing the influence of the different glycaemic meals, as well as the fasting state on the networks during maximal exercise, the benefit of short-term physical activity on the metabolic "environment" and haemostatic balance may be determined. Changes that occur after 30 minutes of recovery from a maximal exercise will give an indication of the possibility of an increased risk after physical activity. An illustration of the hypothesis is given in Figure 1.1.

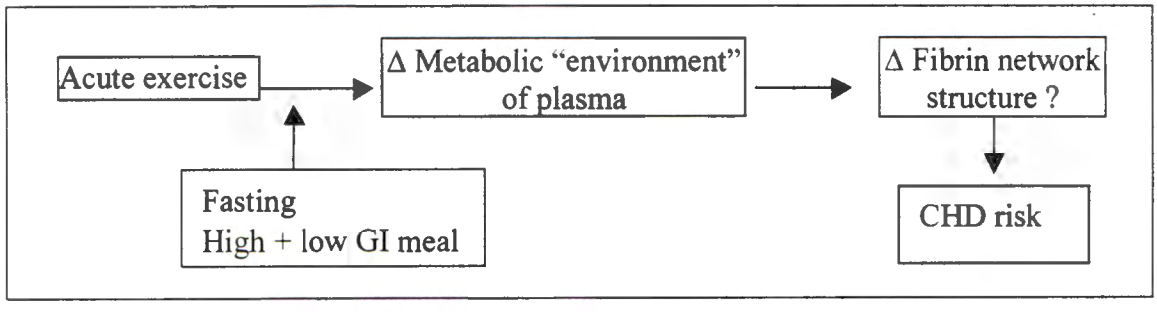

Figure 1.1: An illustration of the hypothesis where an acute exercise or a pre-exercise meal changes the metabolic "environment" that subsequently changes the fibrin network structure.

1.4 Objectives

To test the hypothesis that has been formulated a number of objectives have been defined. These objectives include the investigation of the fibrin network structure of active and sedentary males at rest, maximal activity and 30 minutes of recovery, in order to answer the following questions:

1. What changes are observed in the metabolic "environment" of the plasma of fasting active and sedentary males in response to maximal exercise and 30 minutes of recovery?
2. Does the fibrin network structure characteristics in fasting, active and sedentary males change in response to maximal exercise and 30 minutes of recovery?
3. What influence does a high and low glycaemic index (GI) pre-exercise meal have on the metabolic environment and fibrin network structure 1-hour post ingestion?
4. Do changes occur in the metabolic "environment" and fibrin network structure when sedentary and active males are introduced to a maximal exercise 1 hour after ingestion of a high and low GI meal?
5. What is the response of the metabolic environment and fibrin network structure 30 minutes after the maximal exercise with the pre-exercise high and low GI meal?

1.5 Structure of this thesis

The results obtained with this study will be presented as follows: The introductory chapter will be followed by a review of the relevant literature in Chapters 2 and 3. Chapter 2 will deal with the formation of the fibrin network structure together with the influence of CHD risk factors on the network structure. The literature regarding physical activity and diet on haemostasis will be discussed in Chapter 3. Chapter 4 will give a detailed description of the study design and the methods used in obtaining the data. Chapter 5 will present the results and a discussion of the results in the form of two scientific manuscripts. Finally, the conclusions from this study together with future research proposals and practical implications will be given in Chapter 6.

CHAPTER 2

FIBRINOGEN AND FIBRIN NETWORKS AS CARDIOVASCULAR DISEASE RISK FACTORS

2.1 Introduction

Atherosclerotic vascular diseases are progressive conditions of multi-factorial origin. There is no doubt that atherosclerosis develops as a result of chronic endothelial damage. The endothelium is damaged by chemical or mechanical stimuli, related to various risk factors. These risk factors, categorised in Figure 2.1, include the smoking habit, hypertension, increased blood cholesterol, a high fat diet, oxidative stress, obesity, a lack of exercise and elevated plasma fibrinogen levels (European Atherosclerosis Society, 1993).

In prospective studies, most cardiovascular risk factors correlated with increases in fibrinogen levels (Kannel *et al.*, 1987b; Wilhelmsen *et al.*, 1984). All the mechanisms through which raised fibrinogen levels promote atherosclerosis have not been established. The trauma that atherosclerosis causes to the vascular wall and surrounding tissues, activates the coagulation cascade and formation of thrombin through the intrinsic and extrinsic pathways. Thrombin activates platelets and converts fibrinogen to fibrin. The fibrin fibres eventually interact to form a 3-D network structure (Blombäck, 1996).

Fibrin network structures, or gels, have been studied extensively over the last five decades. Ferry and Morris (1947) classified matrices as ranging between transparent and opaque. Blombäck *et al.* (1994a) have shown that depending on modulating and kinetic factors, fast initial activation leads to tight, rigid gels, while slow activation results in porous, "plastic" gels. Fibrin clots formed in the blood vessels are normally effectively degraded

and removed by the fibrinolytic enzyme system. The existence of abnormal haemostasis, characterised by an imbalance in procoagulant and anticoagulant activities, is now accepted as a major risk factor for atherosclerosis, thrombosis and resultant cardiovascular disease (Tanaka & Sueishi, 1993; Vorster & Venter, 1994).

Since the first results of the Northwick Park Heart study (Meade *et al.*, 1980) were published, there have been increasing reports about the association between haemostatic factors and cardiovascular diseases. Haemostasis depends upon a complicated interplay between plasma coagulation and fibrinolytic factors and inhibitors, blood cells, vessel walls, extra-cellular matrix, as well as haemorheological factors such as blood viscosity and flow (Takada *et al.*, 1994). Disturbances of the haemostatic balance may result in changes in the fibrin network structure with the resulting thrombosis and myocardial infarction or a bleeding tendency. A study by Fatah *et al.* (1996b) indicated that the fibrin network structure, formed *in vitro* in patients with myocardial infarction at under 45 years of age, tend to be tight and rigid and independently associated with the severity of coronary atherosclerosis *in vivo*.

In this Chapter a brief review of CVD risk factors will be given, with a focus on fibrinogen, an abnormal haemostatic balance and fibrin networks as risk factors. The role of physical activity and diet will be discussed in Chapter 3.

2.2 Cardiovascular disease (CVD) risk factors

The Nutrition Research Group (NRG) (2000) recently reviewed and categorised risk factors as either genetic (constitutional) or environmental, including behavioural factors associated with an increased risk of becoming diseased (experiencing chronic cardiovascular disease and/or acute fatal or non-fatal events). The prevalence of a risk factor indicate a person is at risk, but does not necessarily mean than an individual will get the disease (or to experience the cardiovascular event). In individuals risk factors are usually not as strong predictors of disease (cardiovascular events) as are clinical findings of early disease. Risk factors need not be causes of the disease. Causality may be difficult to prove. To establish the relationship of a specific haemostatic variable with the

cardiovascular event there must be a rationally related biology (therefore, a plausible underlying biological mechanism), these should be specifically linked, highly correlated, closely related, temporally coincident and coincidentally "normalised" by appropriate (e.g. antithrombotic) therapy. A risk factor may mark a disease outcome indirectly, by virtue of an association with some other determinants of disease. A risk factor that is not a *cause* of the disease is referred to as a *risk marker*, because it marks the increased probability of the disease.

Not being a cause does not diminish the value of the risk factor as a way to predict the probability of the disease. It implies that removing such a risk factor might not remove the excess risk associated with it.

The risk factors that have been associated with CVD can also be divided into two groups: primary and secondary risk factors (ACSM, 1995). The primary risk factors are those that have been identified as ***causing*** cardiovascular disease, such as hypertension, smoking and hypercholesterolaemia. The secondary risk factors are not less important, but a causal relationship has not yet been established. These factors include diabetes mellitus, stress, obesity and a sedentary lifestyle. CVD is a multifactorial disease, and one risk factor, even optimally controlled, will not completely offset the impact of the others (Gordon & Gibbons, 1991).

The above-mentioned risk factors are all factors that can be managed and changed through a change in lifestyle. Risk factors such as age, family history and gender cannot be changed. Sedentary lifestyle has been regarded as the most prevalent modifiable risk factor, followed by cigarette smoking, elevated serum cholesterol levels and hypertension (Francis, 1996). In Figure 2.1 a summary of the link between the risk factors and cardiovascular disease is given, illustrating possible mechanisms through which environmental risk factors may influence the development of atherogenesis and thrombosis.

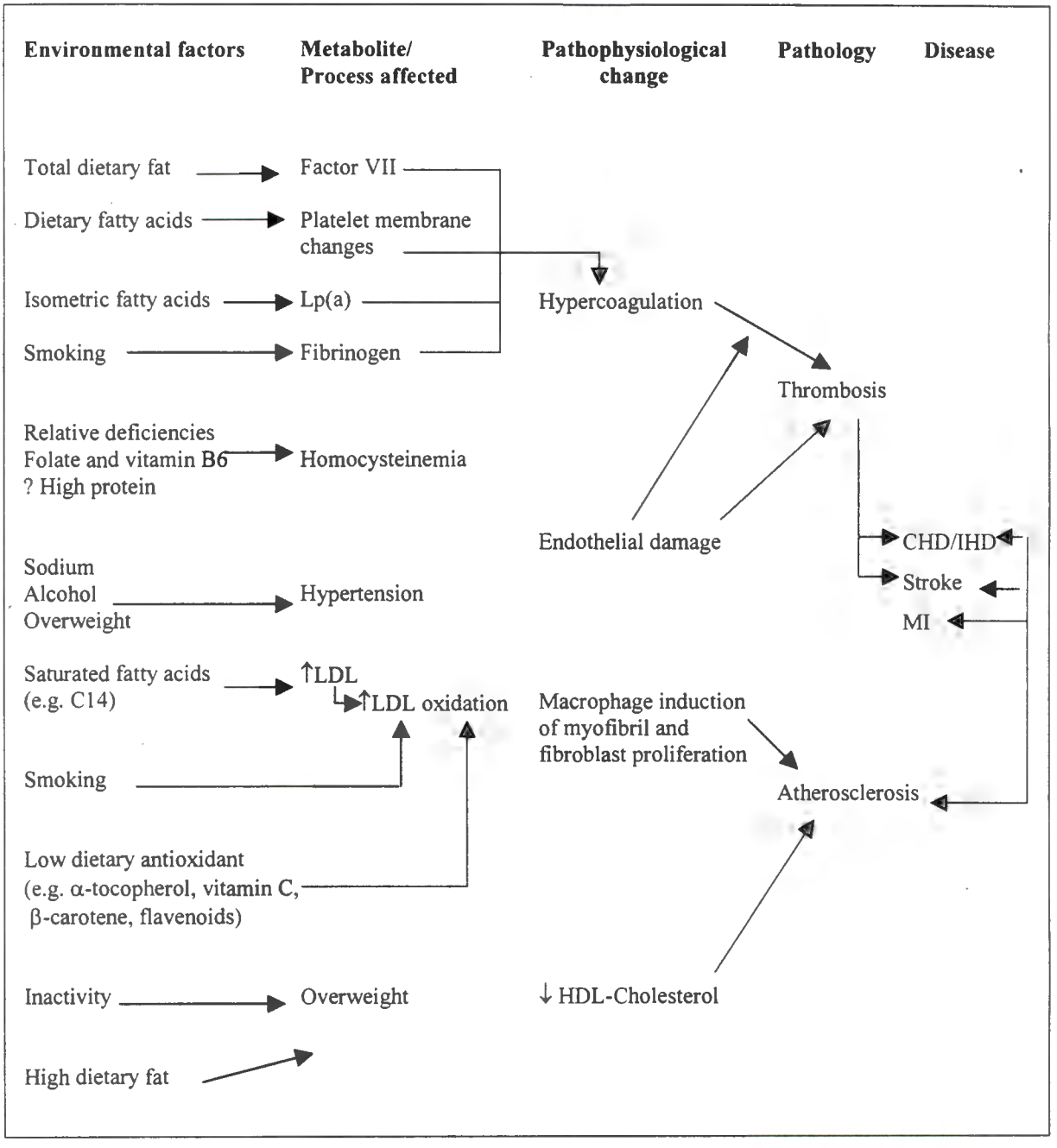

Figure 2.1 A schematic representation of the risk factors and the possible link to CVD (Adapted from Eastwood, 1997)

2.3 Fibrinogen as a cardiovascular risk factor

2.3.1 Introduction

Fibrinogen is a powerful independent cardiovascular risk factor (reviewed by Summaray *et al.*, 1998). The relation was first reported in preliminary results from the Northwick Park Heart Study in 1980 (Meade *et al.*, 1980) and later confirmed in its main findings (Meade *et al.*, 1986). The strength of the association is similar to that of cholesterol or blood pressure. Fibrinogen is strongly associated with mortality (mostly from cardiovascular causes) in patients with intermittent claudication (Banerjee *et al.*, 1992) and with venous thrombosis (Koster *et al.*, 1994).

2.3.2 Fibrinogen as a major risk factor for CVD: the epidemiological evidence

The evidence that fibrinogen is an independent risk factor for CVD has come from 13 prospective population-based studies involving more than 50 000 subjects (summarised in Table 2.1). These collective investigations are of adequate sample size (participants and participants with major cardiovascular events) and composition (randomised, different genetic, occupational, social and geographic backgrounds). Although the study designs differ in follow-up periods, ranging from 5 to 13.5 years, end-point determination and methods of determining fibrinogen, they all demonstrate that fibrinogen is an important, independent risk factor (Ernst, 1993b). In most of these studies, the relationship of fibrinogen with CVD end-points was not dependent on other CVD risk factors such as TC, HDL-cholesterol, LDL-cholesterol and blood pressure. However, effects of smoking, are probably mediated by an increased fibrinogen. The relationship of the CVD risk factors will be discussed in the following section (2.3.3).

These studies have also indicated that various environmental factors lead to an increase in fibrinogen in healthy individuals as summarised in Table 2.2 (adapted from Ernst, 1993b; Meade, 1997).

Table 2.1: The association between plasma fibrinogen and primary cardiovascular disease events: summary of population-based prospective studies

Name of study	Reference	Recruitment age (years)	Duration Follow-up	Number of subjects analysed	Number of events	Type of events recorded	Method for plasma fibrinogen	Results (Data) available
1.Cardiovascular Health Study (Elderly) (CHS)	Tracy *et al.* (1999)	≥ 65	6.5 and 3.5 mean 5	5888 ϕ = 5788	men 602 women 424	Incident CHD, death, stroke/TIA	Von Clauss-method, BLB Fibrometer, Becton-Dickerson	RR for men and women separately, adjusted and crude for incident events, early and late mortality, stroke and TIA (together)
2.Scottish Heart Health Study (SHHS)	Woodward *et al.* (1998)	40-59	8	5095 men 4860 women 9955 men and women	264; total mortality 487	CHD events; coronary death; total death	Von Clauss-method, semi-automatic, Coag-A-Mate X_2, Organon Teknika, Cambridge, UK	HR for men and women separately; age, cotinine, and multiple adjusted for total events, coronary events and all cause mortality.
SHHS	Tunstall-Pedoe *et al.* (1997)	40-59	7.6	5754 men 5875 women 11629 men and women	CHD events 581 CHD deaths 206 Total mortality 591	CHD events; CHD deaths; total mortality		HR and multiplicative constant across consecutive classes for CHD events, CHD deaths, total deaths for men and women separately and age-adjusted
3. Atherosclerosis Risk in Communities Study (ARIC)	Folsom *et al.* (1997)	45-64	5 (4-7)	14477 men and women	CHD incidence events men 238 women 110	CHD incidence events	Thrombin-time titration method, Fibriquick	SRR for men and women separately; crude, age and multiple adjusted; blacks and other separately, age-adjusted.
4. Zutphen Elderly Study	Feskens & Kromhout (1997)	70-89	5	458 men	41	MI	?	Per highest tertile of fibrinogen, unadjusted RR for MI: 1.86 (1.01-3.47); After adjustment #RR=1.78 (0.95-3.40) p=0.08 # NS for stroke.
5. Men born in 1933 study	Rosengren & Wilhelmsen (1996)	50	9	664 men	CHD events 82; CHD deaths 9; All cause mortality 44	CHD events and all-cause mortality	Von Claus-method, Organon, Teknika	RR for CHD events, all cause mortality, crude and multiple adjusted for smokers, non-smokers separately and together.
6. Edinburgh Artery Study (EAS)	Lee *et al.* (1998)	55-74	5	1106 men and women	Upper quartile	Intima-media thickness > 1.05mm in men; > 0.95 in women	Thrombin-clotting time, turbometric, centrifugal analyser (Lowe *et al.* (1991)	OR age and multivariate adjusted, men and women separately.
EAS	Smith *et al.* (1997)	55-74	5	1592 men and women	268	New events		Baseline fibrinogen independently related to risk of stroke (multivariate adjusted) RR = 1.52 (1.17-1.98).
EAS	Lowe *et al.* (1997)	55-74	5	1592 men and women	272 235 45	New events IHD Stroke		RR (95% CI) of stroke and total events for 1SD increase in fibrinogen (0.60 g/L): Total events 1.17 (1.02-1.34) (multiple adjusted) Stroke 1.46 (1.06-2.02) (multiple adjusted).

Table 2.1 (continued)

Name of study	Reference	Recruitment age (years)	Duration Follow-up	Number of subjects analysed	Number of events	Type of events recorded	Method for plasma fibrinogen	Results (Data) available
7. Caerphilly and Speedwell Collaborative Study (CSCS)	Sweetnam *et al.* (1998): comparison of two methods	45-64	5;10	4391 men	533	Incident (major) IHD	Von-Clauss method plus nephelometric (heat precipitation)	Comparison of OR, ϕ nephelometric and Clauss method, unadjusted and fully adjusted at 5 and 10 years
CSCS	Sweetnam *et al.* (1996)	45-63	10	4860 men	603	IHD events		OR, crude and adjusted for age, age + smoking + existing disease, multiple-adjusted.
CSCS	Yarnell *et al.* (1991)	45-63	5.1 and 3.2	4860 men	251	IHD events		Crude RR and OR plus age-adjusted OR.
8. PROCAM (Munster Heart Study)	Assman *et al.* (1998) Junker *et al.* (1997) Assman *et al.* (1996)	40-65 40-65 40-65	8 8 6,8	2691 men 2691 men 2780 men	130 130 130	Major coronary events Major coronary events Major coronary events	Von Clauss method, Behringwerke, Germany	Crude OR and RR men Crude OR and RR men, smokers, non-smokers, adjusted OR without CI 6 and 8 years.
	Heinrich *et al.* (1994)	40-65	6	2116 men	82	Major coronary events		OR and RR, unadjusted.
9. Goettingen Risk, Incidence and Prevalence study (GRIPS)	Cremer *et al.* (1997) Cremer *et al.* (1994)	40-59.9 40-59.9	10 5	5790 men 6002 men	299 109	MI incidence cases MI incidence cases	Nephelometric (immuno-precipitation) Behring.	RR crude and multivariate adjusted RR crude and multivariate adjusted
10. Framingham	Kannel *et al.* (1987)	47-79	12	554 men 761 women	men women	CVD event: CHD attack, stroke, cardiac failure, occlusive event	Clottable, Swain modification of Ratnoff & Menzie	RR and OR, crude, men and women separately and together.
11. Northwick Park Heart Study (NPHS)	Meade *et al.* (1986)	40-64	5 and 5-13.5 mean 6.7	1511 men	105	Major IHD events	Gravimetric, clottable	RR and OR, crude and adjusted for total events, non-fatal MI, IHD deaths, all cause mortality, ≤ 5 and > 5 years
	Meade *et al.* (1980)	40-64	2.75	1510 men	49	Deaths: cardiovascular (27) and cancer (18) and all-cause		RR and OR, crude for cardiovascular mortality
12. Götenborg Study (GS)	Wilhelmsen *et al.* (1984)	54 (in 1967)	13.5	792 men	129	MI and stroke; other deaths	Clottable method of Blömback (1958)	RR and OR, crude
13. Leigh Study (LS)	Stone & Thorp (1985)	40-69		297 men	40	Coronary events: new heart attacks, some secondary; acute sudden death	Nephelometric, heat precipitation	RR and OR, crude

RR: relative risk; HR: hazard ratio; OR: odds ratio; SRR: Standardised relative risk: risk of event with 1 SD increase in plasma fibrinogen (approximately 0.6g/L)

Table 2.2: A summary of the changeable and unchangeable factors associated with increased and decreased fibrinogen (adapted from Meade, 1997; Ernst, 1993b)

Factors associated with increase	Factors associated with decrease
A. Unchangeable factors	
• Age • Gender • Menopause • Heredity • Race • Season (winter)	• Smoking cessation • Physical activity (aerobic) • Moderate intake of alcohol • Body weight normalisation • Postmenopausal hormone replacement • HbsAG gene carriage
B. Potentially changeable factors	• Fibrate drugs
• Smoking • Obesity • Social class • Stress • Oral contraceptives • Pregnancy • Dietary fat • Infection • Diabetes	

2.3.3 Relationship between plasma fibrinogen and other CVD risk factors

Many of the cardiovascular risk factors in the prospective studies correlated with increases in fibrinogen plasma levels (Kannel *et al.*, 1987a; Meade *et al.*, 1986; Stone & Thorp, 1985; Wilhelmsen *et al.*, 1984). It is therefore possible that some cardiovascular risk factors exert atherogenic effects both directly and indirectly via an increase in fibrinogen.

- *Genetics*

According to Hamsten *et al.* (1987) as well as Humphries *et al.* (1987) a genetic determination of fibrinogen levels seems to exist. Differences between individuals of varying genotypes in DNA polymorphisms at the alpha and beta fibrinogen loci have been reported. This was however not confirmed in unrelated individuals or in monocygotic twins (Berg & Kierulf, 1989). More research is needed in this area.

- *Age and menopause*

Most reports have demonstrated that fibrinogen levels increase with age (Meade *et al.*, 1979). Others have found that fibrinogen levels are not age-dependent. This discrepancy could be due to older persons being more prone to disease and infections. When a strict criterion for health is applied, and individuals with hidden diseases are excluded from the analysis, the age-dependence disappears (Kruger *et al.*, 1994).

It has been suggested that age-related changes in coagulation and fibrinolytic factors contribute to the increased risk of atherothrombotic events in postmenopausal women (Kannel *et al.*, 1987b; Hamsten, 1993). Indeed, higher plasma concentrations of fibrinogen (Kannel *et al.*, 1987a) and fibrin D-dimer (Giansante *et al.*, 1994), both markers of thrombogenic risk and reduced endogenous fibrinolytic activity, have been reported in healthy postmenopausal compared to pre-menopausal women.

- *Smoking*

Smoking is known to increase fibrinogen levels in healthy individuals. It represents the strongest known environmental influence on this variable. Snuff-users also have increased concentrations (Eliasson *et al.*, 1991). The effect of smoking on fibrinogen is dose-related (Ernst *et al.*, 1987; Wilkes *et al.*, 1988) and is reversible upon cessation of smoking (Ernst & Matrai, 1987a; Meade *et al.*, 1979). Cross-sectional data showed that following cigarette abstention, a rapid initial decline ensues but levels remain slightly elevated for several years (Meade *et al.*, 1987). This time course would parallel the decline in the risk of ischaemic heart disease after smoking cessation (Dohson *et al.*, 1991). The rapid drop of fibrinogen after quitting smoking sheds doubt on the notion that high fibrinogen levels are a mere reflection of the extent of the underlying atheroma, because regression happens on a different time scale compared with the rapid fibrinogen modification (Meade, 1992).

The Framingham Study provides a valuable, detailed analysis of the interrelation between fibrinogen, smoking and cardiovascular disease (Kannel *et al.*, 1987a; Kannel, 1992). Its authors estimated that 50% of the cardiovascular harm done by chronic smoking is

mediated through its effect of raising fibrinogen. But high fibrinogen levels are associated with an increased cardiovascular risk also in nonsmokers (Yarnell *et al.*, 1991).

- *Hypertension*

In patients with essential hypertension, fibrinogen levels are higher than in normotensive controls (Letcher *et al.*, 1981). Plasma viscosity is elevated in hypertensive subjects, and blood pressure readings is positively correlated with plasma viscosity. Even when hypertension is only mild, patients have higher fibrinogen levels than normotensive controls (Landin *et al.*, 1990). In a large cohort study, the Scottish Heart Health Study, Lee *et al.* (1993) confirmed a strong association of fibrinogen with hypertension.

- *Diabetes mellitus*

Fibrinogen is raised in diabetics. Patients with microvascular involvement and albuminuria have higher fibrinogen levels than diabetics free from such complications (Schmitz & Ingerslev, 1990). Results from the Framingham Study (Kannel *et al.*, 1990), indicated a correlation between blood sugar levels and fibrinogen. Fasting glucose correlates with fibrinogen even in healthy individuals. Ganda and Arkin (1992) showed that fibrinogen is an independent predictor of vascular complications in type II diabetics.

- *Obesity*

In obese individuals fibrinogen is elevated and increases with skinfold thickness. Weight reduction leads to a decrease in fibrinogen concentrations (Rillaerts *et al.*, 1989).

- *Hypercholesterolaemia*

Fibrinogen levels are elevated in patients with familial hypercholesterolaemia (De Minno *et al.*, 1986) and with hypertriglyceridemia (Elkeles *et al.*, 1980). In a large population sample, plasma viscosity correlated positively with total cholesterol and apolipoprotein B (Lp (b)) (Koenig *et al.*, 1989). The baseline data from the GRIPS-study (Cremer *et al.*, 1992) showed that fibrinogen levels are positively associated with total cholesterol, triglycerides, LDL-cholesterol and HDL-cholesterol but not with apolipoprotein A (Lp (a)).

- *Stress*

Fibrinogen is related to social class (Markowe *et al.*, 1985), which has in part been attributed to different levels of emotional stress (Ernst *et al.*, 1986). Tsutsumi *et al.* (1999) have shown in the SHEEP-study that a positive association existed between objective job (stressful) characteristics and plasma fibrinogen concentrations.

2.3.4 Mechanisms through which fibrinogen influence atherogenesis and thrombogenesis

It has not been elucidated whether the increase in fibrinogen levels is responsible for the ischaemic arterial risk or whether it is only a marker of arterial disease (Vasse *et al.*, 1994). Several mechanisms have been proposed to explain the relationship of fibrinogen with CVD.

Fibrinogen and its metabolites affect haemostasis, blood rheology, platelet aggregation and endothelial function (De Minno & Mancini, 1990). A hypercoagulable state would seem to favour the thrombostatic aspects of atherothrombosis. Large amounts of fibrin and fibrinogen have been detected in early arteriosclerotic plaques. Fibrin is thus the principle component of gelatination and fibrous plaques. This could well be fibrinogen's most important role; it certainly is the most obvious. The amount of fibrin formation depends on

the plasma concentration of fibrinogen in experimental (Gurewich *et al.*, 1976; Chooi & Gallus, 1989) and kinetic studies (Naski & Shafer, 1991).

Fibrinogen is the major determinant of plasma viscosity and induces reversible red cell aggregation (Koenig & Ernst, 1992). Both phenomena limit the fluidity of blood. Blood rheology might act by reducing flow, predisposing to thrombosis. Platelet hyperaggregation plays an accepted role in the genesis of atherosclerotic lesions. Fibrinogen binds to receptors on the platelet membrane, which in turn is a precondition for aggregation *in vivo* (Cook & Ubben, 1990). Furthermore, fibrinogen is also integrated directly into arteriosclerotic vascular lesions where it is converted to fibrin and fibrinogen degradation products; it binds low-density lipoproteins and sequesters more fibrinogen (Cook & Ubben, 1990).

Another possible mechanism is that activated monocytes secrete hepatocyte-stimulating factors (HSF) that control the biosynthesis of fibrinogen *via* the hepatocytes. Interleukin-6 (IL-6), leukemia inhibitory Factor (LIF) and oncostatin M (OSM) contribute to HSF activity. Of these factors, OSM induce vascular smooth muscle cells (SMC) to proliferate (Grove *et al.*, 1993). These factors may also lead to fibrinogen being converted to fibrin and eventually fibrin degradation products (FbDP). Fragment E, that is formed from the FbDP, stimulates macrophages and connective tissue cells to produce IL-6 (Smith & Thompson, 1994). The fibrinogen increase could be due to FbDP production by activated monocytes (Vasse *et al.*, 1994). Both fibrin and fibrinogen degradation products have been demonstrated to stimulate smooth muscle cell proliferation and migration (Thompson & Smith, 1989; Smith *et al.*, 1990). A schematic representation of this mechanism can be seen in Figure 2.2.

The fact that fibrinogen is an acute phase reactant deserves careful consideration. Atherosclerosis bears certain similarities to an inflammatory process (Spodick, 1985). For instance, white cell counts are significantly elevated in the presence of active atherosclerosis (Nieto *et al.*, 1992). Thus it would seem theoretically possible that early atherosclerosis itself leads to a mild inflammatory response which slightly elevates acute phase proteins and other variables of the acute phase response. In the absence of more experimental evidence it would be unwise to speculate about the above mechanisms. It is likely that the clinical and epidemiological findings discussed are the result of complex

interactions between these and possibly other phenomena, which we still need to understand more completely.

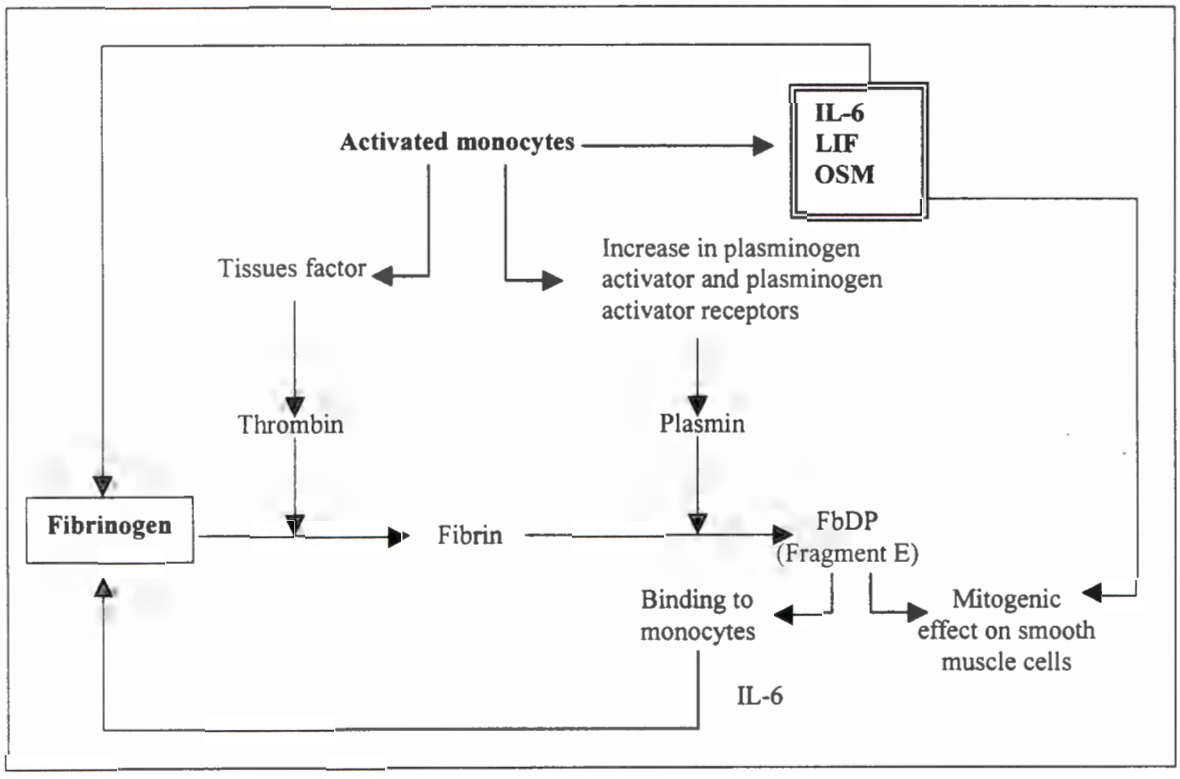

Figure 2.2 A schematic representation of the possible series of events in which activated monocytes are playing an important role both in the development of atherosclerotic lesion and fibrinogen production (Vasse *et al.*, 1994)

2.4 The haemostatic balance

2.4.1 Introduction

In addition to increased plasma fibrinogen, there is a growing perception that a disturbed haemostatic balance because of abnormal levels of a variety of molecules, may be related to atherosclerosis and thrombosis.

The haemostatic system protects the integrity of the vascular system by preventing haemorrhage and promoting tissue defence and repair after injury. Haemostasis is the

culmination of a series of precisely controlled and interrelated actions involving the clotting factors, fibrinolytic enzymes, platelets and the vascular endothelium (Takada *et al.*, 1994). A schematic representation of the interaction by the various parts can be seen in Figure 2.3 (Freese, 1997). The specificity and interaction of molecules from different components of the system, regulated by positive and negative feedback mechanisms carefully control the balance between procoagulant and anticoagulant activities in the system. The balance ensures sufficient blood flow to all tissues and organs under normal circumstances. This interaction of the molecules from the different components of the system also ensures that when the system is triggered by injury, the coagulation activities and subsequent fibrinolysis are localised within the area of injury. Disturbance of haemostatic balance may lead either to thrombosis (clot formation inside the blood vessel) and atherosclerosis, or a bleeding tendency. As mentioned before, haemostasis means the prevention of blood loss. Whenever a vessel is severely ruptured, haemostasis is achieved by several different mechanisms including 1) vascular spasm, 2) formation of a platelet plug, 3) blood coagulation, and 4) growth of fibrous tissue into the blood clot to close the hole in the vessel permanently (Guyton, 1991).

From Figure 2.3 it is clear that the coagulation and fibrinolytic systems interact with each other. The coagulation is initiated to prevent blood loss, with the initiation of fibrinolysis to restore blood supply as soon as possible thereafter (Takada *et al.*, 1994). The role of fibrinogen in haemostasis is to form fibrin network structures around the platelets that adhere to and aggregate around the small lesions in the vessel wall. This platelet plug that is formed leads to the formation of the fibrin network structure that forms the basis of the blood clot. In the following section events leading to the formation of fibrin (clotting) will be briefly reviewed.

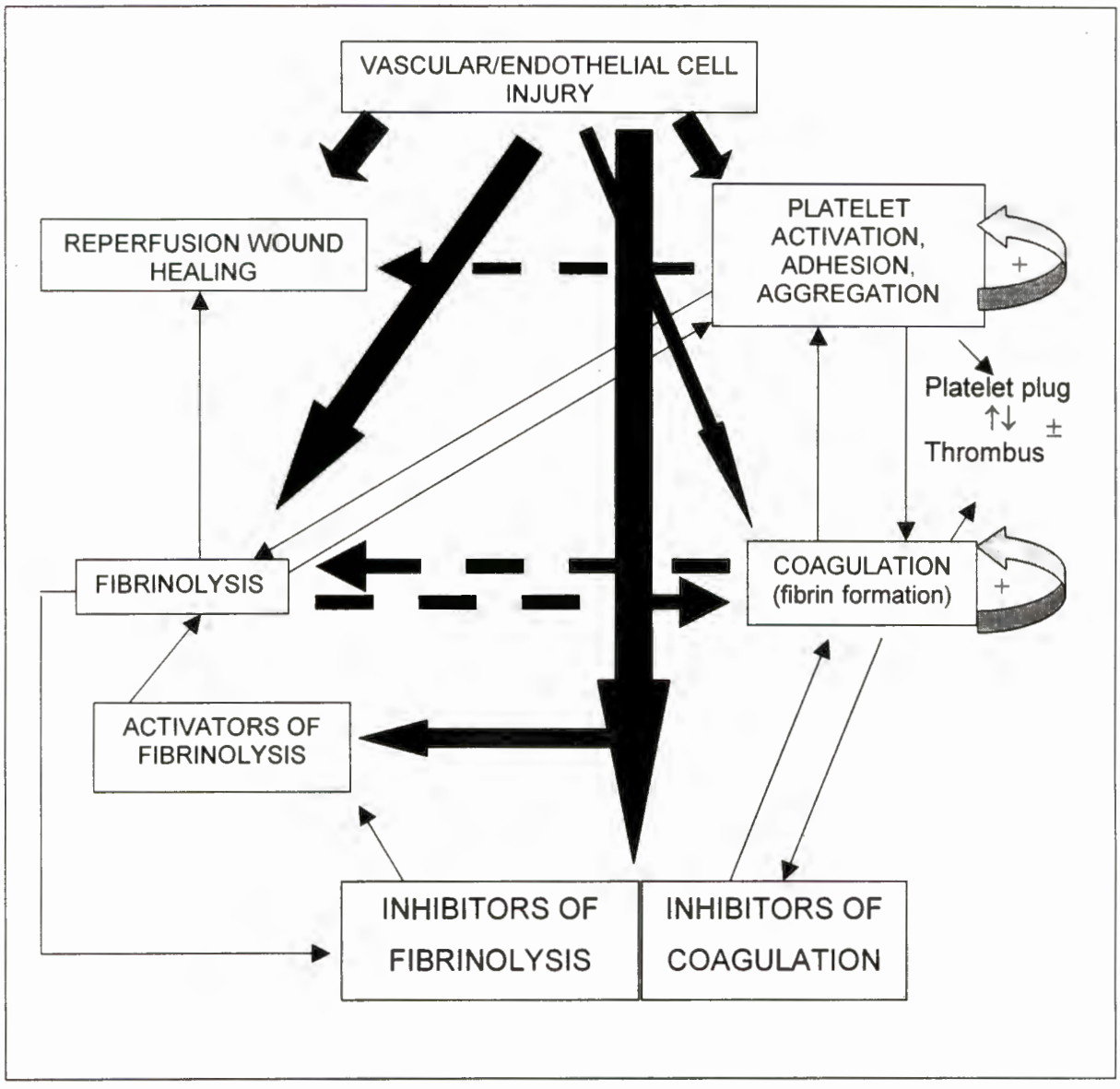

Figure 2.3 **An illustration of how different parts of the haemostatic balance influence the function of other parts (adapted from Freese, 1997)**

2.5 Blood coagulation

Over 40 substances that affect blood coagulation have been found in the blood and tissues, some promoting coagulation (procoagulants) and others inhibiting coagulation (anticoagulants). Whether or not the blood will coagulate depends on the degree of balance between these two groups of substances. Normally the anticoagulants predominate, but when a vessel is ruptured the activity of the procoagulants in that area of damage becomes much greater than that of the anticoagulants, and a clot develops.

Clotting essentially takes place in three steps:

- A substance or complex of substances called prothrombin activators is formed in response to endothelial injury.
- Prothrombin activator catalyses the conversion of prothrombin to thrombin.
- Thrombin acts as an enzyme to convert fibrinogen to fibrin threads that enmesh platelets, blood cells, and plasma to form the clot.

The body has two basic mechanisms by which prothrombin can be activated: a) the extrinsic pathway that begins with trauma to the vascular wall and surrounding tissues, or b) by the intrinsic pathway that begins in the blood itself. In both these pathways a series of different plasma proteins, especially beta globulin, play major roles.

- *The extrinsic mechanism for initiating blood clotting*

This mechanism is activated when trauma occurs to the vascular wall and surrounding tissues. This pathway seems to be more important in the physiological changes in fibrinolysis. Traumatised tissue releases a tissue factor, formerly known as thromboplastin. This transmembrane glycoprotein forms a complex with blood coagulation factor VII, and this complex, in the presence of tissue phospholipids, acts enzymatically on factor X to form activated factor X. The activated factor X forms a complex immediately with the tissue phospholipids released as part of the tissue factor and with factor V to form the prothrombin activator complex. Within a few seconds this complex splits prothrombin to

form thrombin and the clotting process proceeds as schematically indicated in Figure 2.4 (Nemerson, 1995).

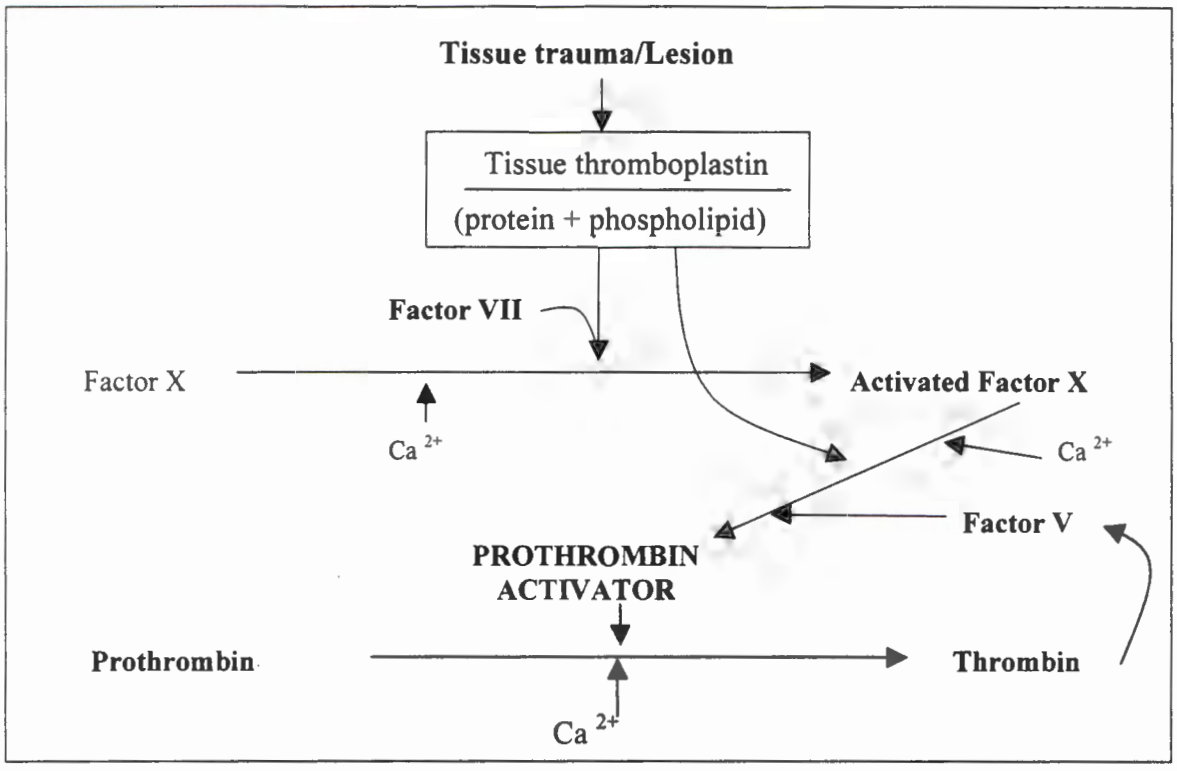

Figure 2.4 A representation of the extrinsic pathway for initiating blood clotting (Guyton, 1986; Nemerson, 1995)

- *The intrinsic mechanism for initiating blood clotting*

This mechanism by which prothrombin activator is formed, is initiated by trauma to the blood itself. The focus of this study will be on this mechanism. Trauma to the blood alters two important clotting factors in the blood – factor XII and the platelets. The intrinsic pathway initiates the activation of factor XII to XIIa in its interaction with a negatively charged foreign surface in the presence of high molecular weight kininogen and prekallikrein (Takada *et al.,* 1994). At the same time, blood trauma also damages the platelets, either because of adherence to collagen or a wettable surface, releasing platelet phospholipids, which play a role in subsequent clotting reactions.

The activated factor XII activates factor XI to activate factor XIa. This reaction also requires HMW kinogen, and it is accelerated by prekallikrein. The activated factor XI then activates factor IX, which in concert with factor VIII and with platelets activates factor X. It is clear that when either factor VIII or platelets are in short supply, this step is deficient.

The following step in the intrinsic pathway is essentially the same as the last step in the extrinsic pathway. Activated factor X forms a complex with factor V and platelet phospholipids that activates prothrombin. The only difference is that the phospholipids in this instance come from the traumatised platelets rather than from traumatised tissues. Prothrombin activator initiates within seconds the cleavage of prothrombin to form thrombin, thereby setting into motion the final clotting step. A schematic representation of the abovementioned intrinsic pathway is given in Figure 2.5 (Guyton, 1986).

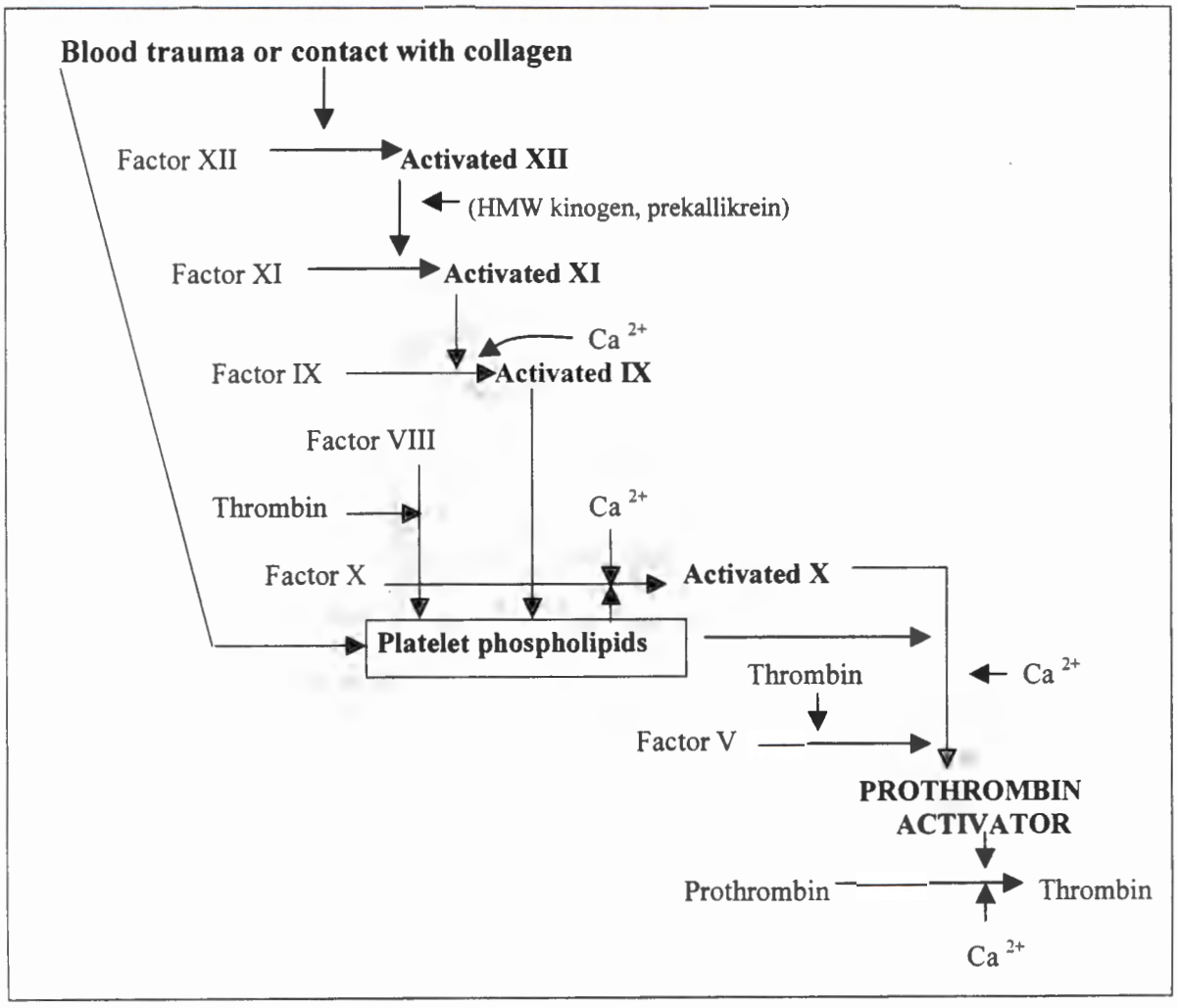

Figure 2.5 **A representation of the intrinsic pathway for initiating blood clotting (Guyton, 1986)**

- *The conversion of prothrombin to thrombin*

Prothrombin is formed continuously by the liver and it is continually being used throughout the body for blood clotting. If the liver fails to produce prothrombin, its concentration in the plasma falls too low to provide normal blood coagulation within 24 hours. The liver for normal formation of prothrombin requires Vitamin K (Olson, 1984). Prothrombin is an alpha globulin, having a molecular weight of 68 700. It is present in normal plasma in a concentration of about 15 mg/dL. It is an unstable protein that can split easily into smaller compounds, one of which is thrombin, which has a molecular weight of 33 700, almost half that of prothrombin.

The rate of thrombin formation from prothrombin is almost directly proportional to the quantity of prothrombin activator complex that is available. The prothrombin activator present, is itself approximately proportional to the degree of trauma to the vessel wall or the blood. Thrombin formation must be in sufficient quantities to overcome the effect of antithrombins and proteinase inhibitors (antithrombin III, protein C, protein S, thrombomodulin and tissue factor pathway inhibitor). During the conversion of prothrombin to thrombin by the prothrombinase complex, prothrombin activation peptide fragment F1+2 (F1+2) is released (Bauer & Rosenberg, 1987). Plasma concentrations of F1+2 are thought to reflect the rate of activation of prothrombin. In a study by Rugman *et al.* (1994) it was found that in 86 randomly selected healthy blood donors, F1+2 correlates more strongly with a wider range of risk factors for IHD than either fibrinogen or factor VII. For the conversion to take place, calcium ions have to be present.

Thrombin is a multifunctional molecule. In addition to fibrinogen catalysis, it initiates fibrinolytic activity by stimulating the release of tissue plasmin activator (t-PA) from endothelial cells (Kitaguchi *et al.*, 1979; Levin *et al.*, 1984). Together with thrombin, antithrombin (AT) is the main inhibitor of the plasma coagulation process. Research by Elgue *et al.* (1994) indicated that plasma AT regulates the rate of prothrombin-thrombin conversion, the clotting time and the formation of the fibrin gel.

2.6 Fibrin network formation

2.6.1 Introduction

Formation of fibrin networks is initiated by the enzymatic conversion of fibrinogen to fibrin by thrombin. In order to understand the position of the fibrin network formation in the haemostatic "balance", a schematic representation of this balance between clotting and fibrinolysis is given in Figure 2.6.

The formation of the fibrin network structure is influenced by modulating and kinetic factors that result in changes in the architecture of the networks (Blombäck, 1994). These differences in the network structure can be determined by various techniques (Blombäck *et al.*, 1994b; Nair *et al.*, 1991a; Dhall *et al.*, 1976) that describe the strength, porosity and rigidity of the networks. Grossly abnormal fibrin network structures appear to be associated with coronary atherosclerotic disease (Blombäck *et al.*, 1994a; Fatah *et al.*, 1996b).

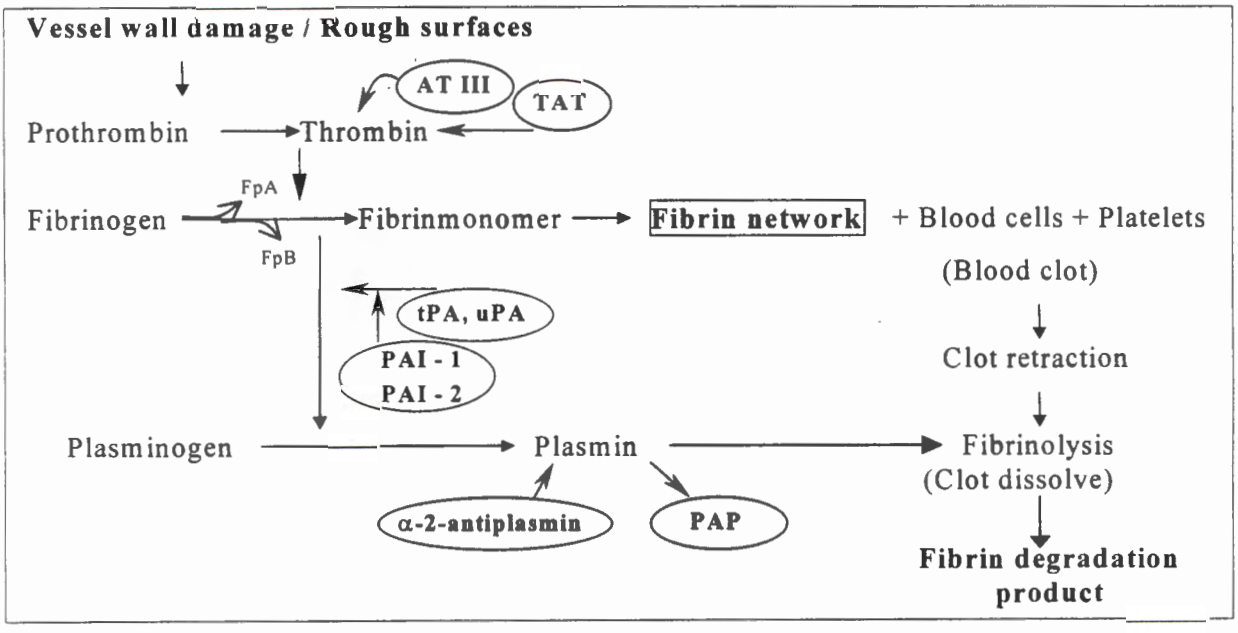

Figure 2.6 A schematic representation of the coagulation and fibrinolytic mechanism (Adapted from Vorster *et al.*, 1997)

2.6.2 Fibrinogen: the substrate

Fibrinogen, synthesised in the liver, has an estimated molecular weight of 340 kDa (Caspary & Kekwick, 1957), with an average 3g/L plasma concentration. Fu and Grieninger (1994) described a new fibrinogen form in plasma, (Fib_{420}) that has an estimated molecular weight of 420 kDa. This variant occurs in homo-dimeric form in plasma and constitutes 1 – 2% of the total fibrinogen pool. In this study the properties of the 340 kDa molecule will be described.

Hydrodynamic measurements indicate that the fibrinogen molecule is a rod-ellipsoid shaped particle, having a length of 40 – 50 nm and a length-width ratio of 5 to 10 (Scheraga & Laskowski, 1957). Studies have indicated that the molecule might contain as much as 6g of water per gram of protein. This high degree of hydration suggests that the molecules have a swollen lattice-like oblate or rod-like structure (Blombäck, 1996).

Fibrinogen is a tridomainal (two outer D domains and a central E domain) disulphide-bridged molecule. The molecule is a symmetric dimer of which the two halves consist of three polypeptide chains, termed Aα, Bβ, and γ (Mosesson & Finlayson, 1976; Doolittle, 1983; Henschen *et al.*, 1983). The two halves are covalently joined in the central amino-terminal E domain by five disulphide bridges, two between γ8 and γ9, another at Aα28, and two between Aα36 and Bβ65 (Blombäck, 1970; Blombäck *et al.*, 1976; Huang *et al.*, 1993; Zang & Redman, 1994). Disulphide bridges form the two symmetrical halves. A reciprocal bridge forms between the γ8 and γ9 position, thus orientating γ chains in an antiparallel direction (Hoeprich & Doolittle, 1983). This arrangement is consistent with electron micrographs of appropriately disposed molecules showing a 2-fold axis of symmetry of D domains perpendicular to the long axis (Mosesson *et al.*, 1981; Méndez *et al.*, 1996). The existence of a symmetrical disulphide bridge at Aα28 suggests that the amino-terminal regions containing the fibrinopeptide A (FPA) sequence are near one another or are possibly even aligned in the same direction (Mosesson, 1998).

The structural model of fibrinogen presented in this study, is based on the work done by Mosesson and his co-workers as illustrated in Figure 2.7 (Mosesson *et al.*, 1995; Siebenlist *et al.*, 1995). It differs from other models, particularly with respect to the location of two

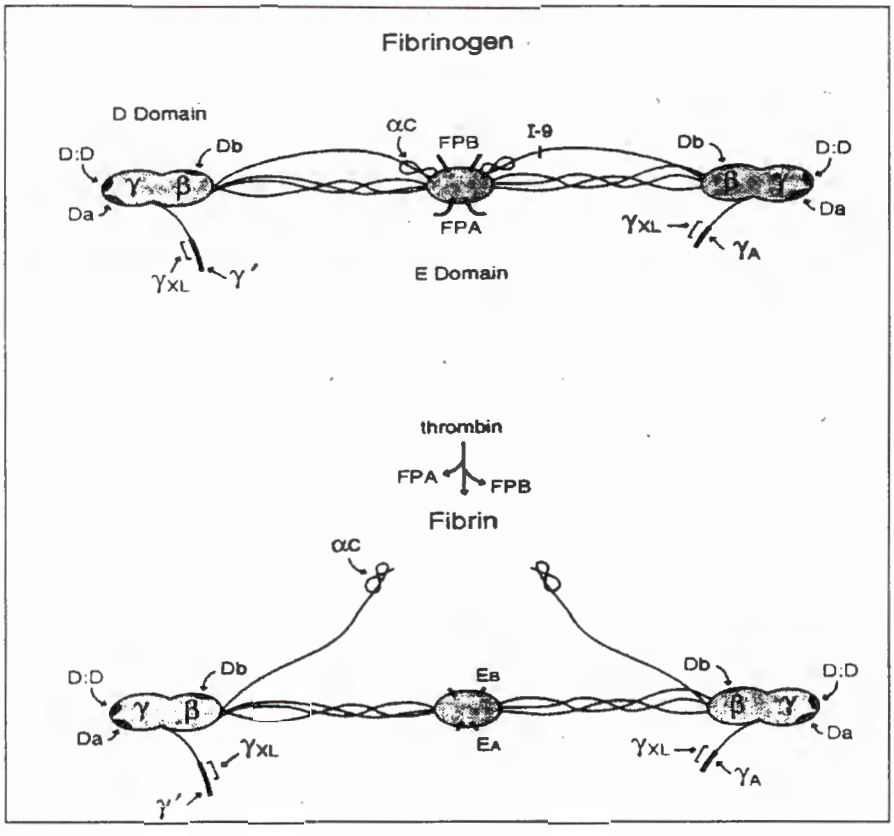

Figure 2.7 **A schematic model of fibrinogen indicating the major domains with the release of fibrinopeptide A and B after interaction with thrombin (Mosesson, 1998)**

newly defined constitutive self-association sites in the fibrinogen D domain, namely the D:D site at the outer end of the D domain and the γ_{XL} crosslinking site, which emerges from the middle of the D domain. The γ_{XL} overlaps and is part of the γ chain crosslinking site. The other, D:D is located at the outer end of each D domain. The existence of D:D at this outer site is consistent with other reported results (Shainoff & Dardik, 1983; Rozenfeld & Vasileva, 1991). Findings indicated that D:D site interactions are necessary for the proper linear alignment of fibrinogen or fibrin molecules in assembling polymers.

In order to initiate coagulation, thrombin (EC 3.4.21.5) and fibrinogen have to interact. This interaction leads to the formation of fibrin (Brass *et al.*, 1976). Many workers have studied the kinetics of this reaction over the previous decades (Steiner & Laki, 1951). Thrombin is a trypsin-like enzyme, which on protein substrates has a narrow specificity.

Of the few hundred trypsin-sensitive bonds in the fibrinogen molecule, only four are preferentially cleaved by thrombin, resulting in the release of 2 fibrinopeptide A (FPA) and 2 fibrinopeptide B (FPB) molecules (Blombäck, 1996) discussed in 2.6.3. Although thrombin and factor XIIIa catalyse the formation of fibrin and its cross-linking to α_2-antiplasmin, activated protein C (APC) inhibits fibrin formation by regulating thrombin generation (Marlar *et al.*, 1982). Research by Gruber *et al.* (1994) led to the hypothesis that APC, *in vivo*, may enhance the efficacy of thrombolysis by altering the internal matrix structure of maturing thrombi and by inhibiting deposition of new fibrin on thrombi.

2.6.3 The splicing of FPA and FPB from fibrinogen

In all animal species studied fibrinogen-fibrin transformation takes place after removal by thrombin of two acidic peptides, fibrinopeptide A and fibrinopeptide B from the N-terminal portion of the α and β-chains, respectively. The release of these peptides activates the fibrinogen molecule. Of the few hundred trypsin-sensitive bonds in the fibrinogen molecule, only four are preferentially cleaved by thrombin, resulting in the release of two fibrinopeptide A (FPA) and two fibrinopeptide B (FPB) molecules. Fibrinopeptide A is always released at the fastest rate. (Blombäck *et al.*, 1978). It seams that fibrinopeptide A needs to be released for the activated molecule to be incorporated in the growing polymer. The product of the proteolysis is the formation of a fibrin monomer.

The process is started with the cleavage of FPA (Aα1 to 16) from the amino-terminal region of each Aα-chain. Fibrin assembly is initiated by exposing a polymerisation site in the E domain termed E_A. Each fibrin E_A site subsequently combines with a constitutive complementary site (Da) in the D domain of neighbouring molecules (Kudryk *et al.*, 1973; Olexa *et al.*, 1981) that is situated in a carboxy-terminal γ chain segment encompassed by γ337 - 379 (Shimizu *et al.*, 1992). This way a molecule of fibrin monomer is formed that has the automatic capability of polymerising with other fibrin monomer molecules. The molecules combine in a half-staggered fashion. This is because the interacting sites are in the centre and end positions, respectively (Blombäck, 1991).

Fibrinopeptide B is released by thrombin at a slower rate than fibrinopeptide A. A second set of polymerisation sites is now becoming operative. The release of fibrinopeptide B (FPB) from polymeric nuclei appears to allow for a higher functionality. This means that polymerisation in different spatial directions commences and gelation occurs at an earlier stage of activation than does when only fibrinopeptide A is released. Release of fibrinopeptide B thus serves as a switch to speed up gelation and minimise the concentration of soluble polymeric species that otherwise would be required to induce gelation (Blombäck, 1991). In a case study of a 50-year old person with an antibody defect that delayed thrombin-mediated fibrinopeptide B release, a prolonged prothrombin and thrombin time was found (Nawarawong *et al.*, 1991). This confirmed the results that the release of fibrinopeptide B leads to the speeding of the clotting time. The release of fibrinopeptide B is accompanied by increased calcium ion binding and pH shift (Mihalyi, 1988). In Figure 2.8 a schematic representation of the changes to the fibrinogen molecule due to thrombin interaction is illustrated.

2.6.4 Fibrin assembly

Initial fibrin assembly involves Da:EA associations that result in the formation of double-stranded fibrils in which fibrin molecules become arranged in an end-to-middle domain staggered in an overlapping manner (Hantgan *et al.*, 1983). The fibrils underfold branching and lateral fibril associations that result in a network of fibres (Carr *et al.*, 1977; Mosesson *et al.*, 1989). Two mechanisms account for the branched structures that constitute a three-dimensional fibrin matrix as illustrated in Figure 2.8. The first type of branch consists of a pair of double-stranded fibrils that converge to form a "tetramolecular" branch point. This type of lateral fibril association also accounts for the formation of thick fibre bundles. The second type of branch junction, termed the "trimolecular" branch point, constitutes the junction of three double-stranded fibrils. Trimolecular branching probably plays an important, possibly dominant, role in the initial and ultimate structuring of a three-dimensional fibrin matrix (Mosesson, 1998).

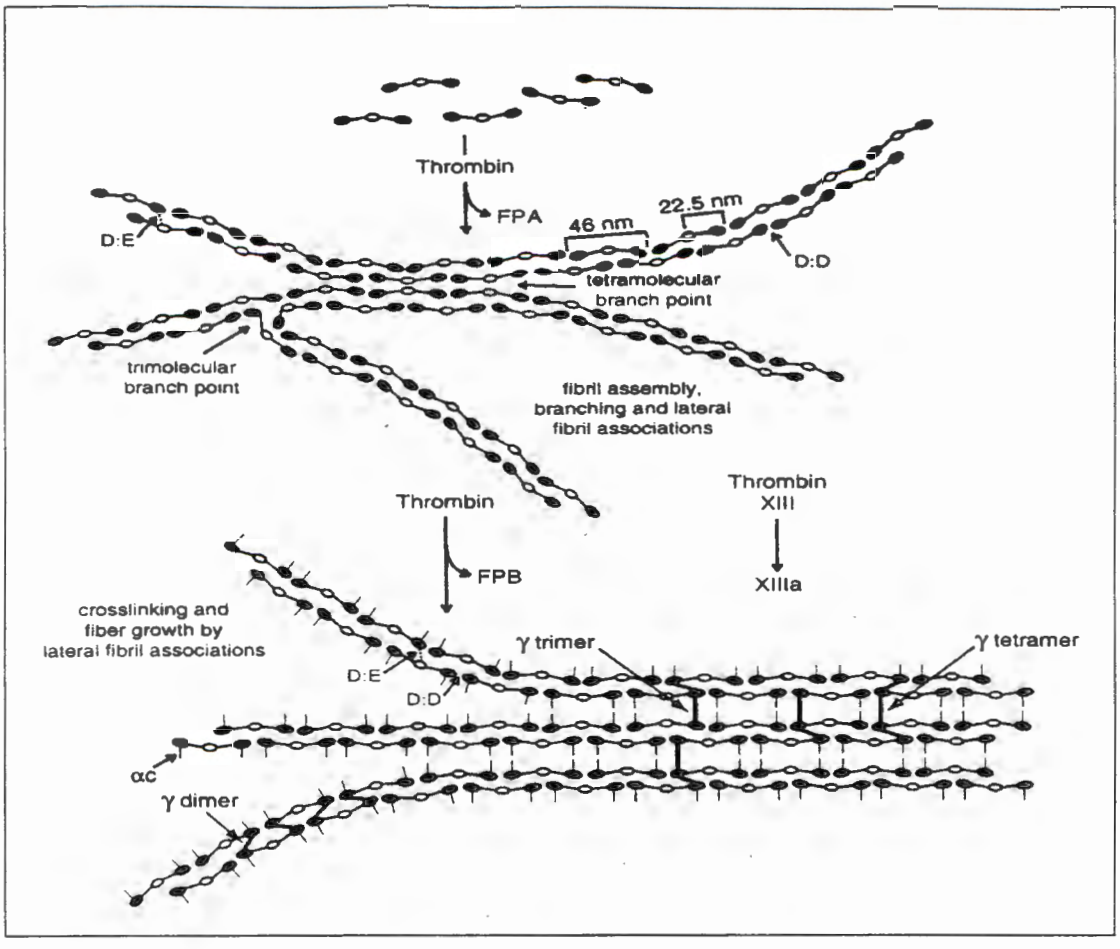

Figure 2.8 An illustration of fibrin polymerisation and lateral association together with crosslinking (Mosesson, 1998)

As mentioned previously, FPB release occurs more slowly than the release of FPA and exposes an independent polymerisation site, E_B (Shainoff & Dardik, 1983). The E_B site is utilised through interactions with a complementary Db site located in a carboxy-terminal β-chain segment of the D domain. The E_B:Db interaction is not absolutely required for lateral fibril and fibre association, but it contributes to this process through co-operative interactions resulting from alignment of D domains in the fibrin polymer. Therefore, many fibrin monomer molecules polymerise within seconds into long fibrin threads that form the reticulum of the clot. In the early stages of polymerisation the fibrin monomer molecules are held together by weak non-covalent bonds, mostly hydrogen and/or hydrophobic, and the threads also are not cross-linked with each other; therefore, the resultant clot is weak and can be broken apart with ease.

However, still another process occurs during the following few minutes that greatly strengthens the fibrin reticulum. This involves a globulin, fibrin-stabilising factor, that is normally present in small amounts in plasma but is also released from platelets entrapped in the clot. Before fibrin-stabilising factor can have an effect on the fibrin threads it must be activated. Thrombin that causes fibrin formation also activates fibrin-stabilising factor. This activated substance operates as an enzyme to cause covalent bonds between the fibrin monomer molecules as well as multiple cross-linkages between the adjacent fibrin threads, thus adding tremendously to the three-dimensional strength of the fibrin network structure. The formed fibrin latticework entraps blood and platelets to form the red blood clot.

Crosslinking of fibrin involves fibrin undergoing intermolecular crosslinking by forming ε-(γ-glutamyl)-lysine iso-peptide bonds (Pisano *et al.*, 1968) in the presence of factor XIIIa. These bonds are formed in a reaction between a proton acceptor glutamine residue in one chain and a proton donor lysine residue in another chain with concomitant release of ammonia (Pisano *et al.*, 1972). Crosslinking of fibril γ-chains involves formation of γ-dimers (Chen & Doolittle, 1969) which occur as reciprocal bridges between lysine at position 406 of one γ-chain and glutamine at position 398 or 399 of another (Chen & Doolittle, 1971). Slower intermolecular crosslinking among α-chains creates oligomers and larger α-chain polymers (Schwartz *et al.*, 1971). Crosslinking also occurs between α-chains and γ-chains (Shainoff *et al.*, 1991). Research has indicated that in addition to γ-dimers, higher-order forms of crosslinked γ-chains occur, namely γ-trimers and γ-tetramers (Siebenlist & Mosesson, 1992). Because there is only one donor lysine residue at γ406 (Purves *et al.*, 1987), it is safe to assume that trimeric and tetrameric cross-linked structures are formed by utilisation of that same residue. The crosslinking pattern of these multimeric chains differs from the simpler reciprocal-bridging pattern that characterises γ-dimers. Whereas γ-dimers originate at bimolecular junctions within double stranded fibrils, γ-trimers and γ-tetramers arise mainly at interfibrillar junctions or possibly at branch points (Mosesson, 1998).

Experiments suggest that the initial fibrin polymers, whether cross-linked or not, create nuclei for growth of linear polymers in different directions in space. A network containing water is thus formed. This is the fibrin gel or fibrin network structure. Light-scattering studies of fibrin polymers, proceeding gelation, showed different assembly patterns of

fibrin, known as fibrin I and fibrin II. Lateral aggregation of protofibrils are more pronounced in fibrin II assembly (Hantgan & Hermans, 1979; Weisel *et al.*, 1993).

Blood clots are composed of a meshwork of fibrin threads, running in all directions and entrapping blood cells, platelets, and plasma. The fibrin threads adhere to damaged surfaces of blood vessels; therefore, the blood clot becomes adherent to any vascular opening and thereby prevents blood loss.

2.6.5 The architecture of the fibrin network structure

The initial structure is a long thin protofibril only two monomer units wide (Hantgan & Hermans, 1979). As the protofibrils reach a critical length, they begin to align themselves along their major axis resulting in larger fibres composed of bundles of protofibrils (Hantgan *et al.*, 1983). A given protofibril may be involved in one fibre along one portion of its length while being part of another fibre along another portion. As fibres continue to increase in size, virtually all the available fibrin monomers are incorporated into the network. The result is a space-filling structure composed of fibrin fibres separated by relatively empty space (Carr *et al.*, 1977). In plasma, like in purified fibrinogen-thrombin systems, kinetic and modulating factors determine the structure of the fibrin network structure.

The kinetic factors are thrombin and fibrinogen concentrations. The modulating factors affect the structures as they are being formed with otherwise constant kinetic factors. This leads to the formation of either long, thin fibres or short, thick fibres as represented in a schematic illustration in Figure 2.9. Such factors are proteins like albumin, fibronectin, specific ions like calcium and the total ionic strength (Blombäck *et al.*, 1994b). Low ionic strength, low pH, and the presence of divalent cations favour thick fibre formation (Blombäck, 1996). Some cellular release products such as leukocyte cationic protein, and platelet factor 4 also favour thick fibre formation.

Plasma proteins have variable effects. It is likely that the plasma proteins act by volume exclusion (Laurent, 1995). This is supported by the fact that dextran has a similar effect to albumin (Blombäck *et al.*, 1990). This will lead to spatial restrictions of polymerisation

processes. Coarser and more porous network structures will be formed. Albumin, which comprises some 50 - 60% of total plasma protein, reduces fibre diameter at low concentrations, which leads to the formation of tight network structures composed of fine fibre strands (Galanakis *et al.*, 1987).

Binding of calcium to low-affinity binding sites also has a pronounced effect on the porosity of the fibrin network structure (Okada & Blombäck, 1983). The porosity and strand width increase with increasing calcium concentration. Marx (1988) indicated that Zn-ions, like calcium ions, have similar effects on the fibrin network structure.

Fibronectin, a plasma protein known to bind to fibrinogen, was investigated by Nair and Dhall (1991) for its influence on the fibrin network structure. The fibronectin incorporation into fibrin is catalysed by factor XIII and is a satiable process. Consequently, the fibre strands will increase in width and the turbidity of the matrix will increase. Because the fractional volume of fibronectin in the network is small, the porosity decreases only slightly.

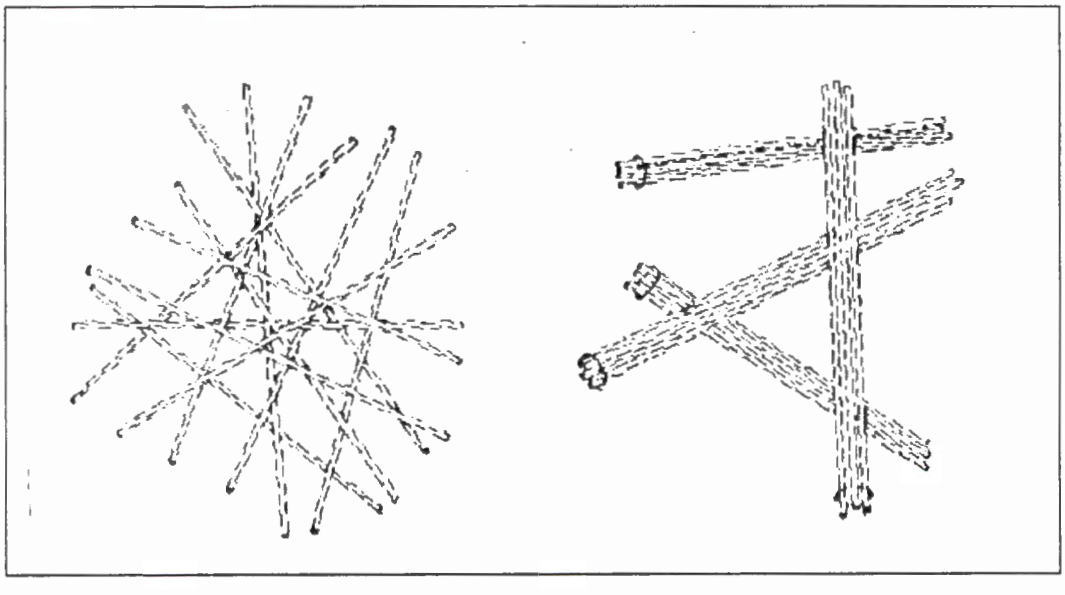

Figure 2.9 **An illustration of the variation of pore size with gel fibre diameter. Left represents a gel composed of thin fibres with right representing a gel composed of thick fibres. The fibre mass is the same in both gels. When the mass is concentrated into thick fibres, spaces and pores between the fibres increase in size (Carr & Hardin, 1987)**

Causes of altered plasma fibrin fibre structure include elevated immunoglobulin levels, fibrin polymerisation inhibitors such as fibrin degradation products, dysfibrinogenaemias - either primary or secondary to hepatic insufficiency, and interference by drugs such as hydroxy-ethylstarch and dextran. Given the dependence of fibrin structure on its micro-environment, additional clot altering variables will undoubtedly be recognised as fibrin structural analysis become routinely available. The common feature of a normal fibrin network structure is an ordered network composed of straight rod-like fibre elements which cross each other in space and often originate in nodes (Blombäck *et al.*, 1989). Increasing the kinetic factors will result in tighter, less porous networks with thinner fibres and higher density of nodes. These structures are supposedly more rigid, since flow of liquid through the gels is impaired. Conversely, low concentrations of kinetic factors result in porous networks with thick fibres and fewer nodes as illustrated in Figure 2.9. The faster the activation the larger the density of polymeric nuclei and the tighter the gel structure (Blombäck, 1991). This is an indication of the alterations that the micro-environment is capable of producing on fibrin assembly and fibre structure. Malan (1999) compiled a detailed discussion on the factors influencing the formation of the fibrin network structure. The consequences of the different fibrin network structures will be discussed at a later stage in section 2.6.8.

2.6.6 Methods for the determination of fibrin network structure

Although fibrin network structures have been extensively investigated since the pioneering work of Ferry and Morrison in 1947, much of the work has concentrated on the biophysics of network development. Investigations into the role of fibrin network structure in haemostatic disorders, in thrombosis and other clinical areas have been hampered on account of lack of suitable methods for study (Nair *et al.*, 1991a). Fibre size can be measured by gel perfusion or by turbidity techniques. The larger and more extended a molecule, the more light it will scatter. Thus gel turbidity is a function of fibre thickness. Advantages of turbidity measurements include simplicity, and relative ease of interpretation. A major disadvantage has been the requirement of a scanning spectrophotometer. By turbidity measurement one can obtain a measure of the average fibre mass-length ratio and fibre diameters of the hydrated fibres in fibrin gels (Carr & Hermans, 1978). Carr and Zekert (1990) have demonstrated that fibre mass-length ratio

are a linear function of turbidity at a given wavelength. Knowledge of a gel optical density at one wavelength is thus adequate to determine turbidity (μ_T).

The measurements of permeability of fibrin network structure give a measurement of porosity (Blombäck *et al.*, 1990). The permeability of clots is a function of pore size, which can be directly measured by perfusing liquid through the fibrin network structure. Pore size can also be estimated as a function of fibre size. If the amount of fibrin in a given volume remains constant, pore size will increase as fibre size or mass-length ratio increases. When fibrin is concentrated in a few large fibres, the distances or voids separating them will be increased.

Additionally to calculating the mass-length ratio and permeability, the fibre density (Carr & Hermans, 1978), fibrin content (Van Gelder *et al.*, 1996) and tensile strength (Nair & Shats, 1997) of the fibrin fibres can be determined.

2.6.7 Fibrinolysis of the formed blood clot

Within a few minutes after a clot is formed, it begins to contract and usually expresses most of the fluid from the network within 30 to 60 minutes. The network becomes more contracted due to the platelets activating the actin and myosin molecules. This helps to compress the meshwork into a smaller mass. In order for fibrinolysis to occur, plasminogen has to be activated by tissue plasminogen activator (t-PA) to plasmin and be in intimate contact with the fibrin surface (Wallen, 1977). Within a day or two after blood has leaked into a tissue and clotted, these activators usually cause the formation of enough plasmin that it in turn begins to dissolve the fibrin network structure. When a clot is formed, a large amount of plasminogen is incorporated in the fibrin network structure along with other plasma proteins. However, this will not become plasmin and will not cause lysis of the fibrin network structure until it is activated. Tissues and blood contain substances that can activate plasminogen to plasmin, including a) thrombin, b) activated factor XII, c) lysosomal enzymes from damaged tissues, and d) factors from the vascular endothelium.

Tissue type (t-PA) a single chain protein (70 kDa) that is synthesised in the endothelial cells, and urokinase type (u-PA) are two types of activators that convert inactive plasminogen to active plasmin. The adsorption of t-PA and plasminogen on the fibrin fibre surface allows the necessary contact needed to affect optimal activation rates for plasmin production and spatial proximity of the fibrin substrate. Fibrin plays a dual role in this series of events. It does not only enhance t-PA activation, it also acts as substrate for plasmin (Gabriel *et al.*, 1992).

Fibrinolysis is initiated by active t-PA binding to the fibrin surface of a thrombus and activating plasminogen to plasmin. t-PA exists in two forms in plasma, namely, active t-PA and t-PA complexed to PAI-1 and other inhibitors. The t-PA activity assay measures functional t-PA, while the total t-PA antigen assay measures active plus complexed t-PA. Prior studies have also shown that as PAI-1 activity increases, the total amount of t-PA in the blood also rises. In resting samples, high PAI-1 activity is associated with high total t-PA antigen but low t-PA activity. The binding site for t-PA and plasminogen is on the E domein (Weitz *et al.*, 1991).

The plasmin rapidly cleaves the bonds in the C-terminal portions of Aα-chain. Bonds are also cleaved in both the N-terminal and C-terminal portions of Bβ-chains. The γ-chain is also hydrolysed by plasmin, but at a rate considerably slower than that for the other two chains (Gaffney, 1977). This gives rise to the final products of the digestion that are the D and E fragments together with a number of smaller fragments. One mole of fibrinogen gives 2 mol of D and 1 mol of E. The fibrinolytic process is further regulated and influenced by the concentrations and activities of α-2-antiplasmin, plasminogen activator inhibitors (PAI-1 and PAI-2) which act as inhibitors for t-PA, as well as thrombin and fibrin itself (reviewed by Van der Bom *et al.*, 1996).

The 2 mol D described above was named D-dimer and is partly characterised as two D fragments from adjacent fibrin units of clotted fibrin and linked by residual factor XIII mediated crosslinks between the γ-chain remnants in the two D fragments (Graeff *et al.*, 1979). The D-dimer is a quantitative measurement of fibrin degradation products that are formed during the lysis of the fibrin network structure.

The types of fibrin network structure that is formed also influence fibrinolysis. Fibrin network structure has been implicated in determining the number of t-PA-binding sites on fibrin (Ping & Gaffney, 1991) and resistance to plasmin attack. Ping and Gaffney (1991) have observed that production of thick fibrin fibres is associated with adsorption of more t-PA and faster fibrinolytic rates. Results of a study performed by Gabriel *et al.* (1992) showed that plasmin production by t-PA activation of plaminogen is proportional to the fibrin fibre thickness, which may provide greater surface for assembly of the fibrinolytic complex. As the concentration of plasmin converted is increased, the rate of fibrinolysis is expected to escalate. With thin fibre production, a decrease in the plasmin concentration and slower fibrinolytic rate is expected. In addition to a decrease in t-PA binding site on thin fibrin fibres, a decrease in plasmin-binding sites may also exist. This hypothesis was formed when much slower fibrinolytic rates occurred when plasmin was substituted for t-PA and plaminogen. Gabriel *et al.* (1992) concluded that decreased fibrinolytic rate is not only a result of a slower production of plasmin for thin fibres, but also that thin fibres are more resistant to plasmin digestion than thick fibres.

The consequence of the different types of fibrin network structures that are formed has a direct influence on the occlusive power of these structures. This means that the common denominator of factor VII activity and fibrinogen concentration as risk factors in ischaemic heart disease (IHD) may be the architecture of an established clot network rather than the mass of deposited fibrin (Blombäck, 1996).

2.6.8 The association of fibrin network structure with cardiovascular disease

Fibrin network structure is known to be altered in disease states (Nair *et al.*, 1993). It has been demonstrated that in peripheral vascular disease (PVD), diabetes, hypercholesterolaemia (Nair *et al.*, 1993) and myocardial infarction (Fatah *et al.*, 1992) fibrin networks are composed of thin fibres with increased tensile strength. This network has significantly reduced permeability and is highly resistant to lysis. The coagulation mechanism is normally active at a low level, even without tissue injury. Small elevations in coagulation factor activities and concentrations result in a hypercoagulable state (Miller, 1992), associated with increased atherosclerosis and thrombosis. Raised fibrinogen concentration and factor VII coagulant activity (VIIc) are the two clotting factors that have

been identified in epidemiological and clinical studies as risk factors for cardiovascular disease (Mennen *et al.*, 1996).

Studies have established that the clotting potential and fibrinogen concentration acting in concert determine the structure of fibrin network structures. This suggests that the common denominator of factor VII activity and fibrinogen concentration as risk factors in IHD may be architecture of an established clot network rather than the mass of deposited fibrin. Fibrin always occupies a small part (less than 1%) of the network volume. The volume filling capacity of the fibrin network structure is mainly due to water retention. The resiliency of this structure would determine the occlusive power of it. Tight network structures with small pores and thin fibrin strands are rigid. They are also brittle and during strain rather break than give off the water trapped in the network. These networks are considered thrombogenic. Fibrin network structure with thicker strands and large fibre meshes give off water when strained; they deform and synergise. These clots are non-thrombogenic. Increase in the clotting potential or fibrinogen concentration favours formation of thrombogenic types of fibrin network structures (Blombäck, 1996).

In a study by Fatah *et al.* (1992) a relationship between fibrin network structure and IHD was investigated. Fibrin network structures formed from plasma of men that had suffered myocardial infarction before the age of 45 years were compared with those of healthy men. It was found that the studied subjects presented a proneness to formation of tight, rigid and space-filling fibrin network structures with small pores. These tight, rigid and space-filling fibrin network structures thus appear to be associated with premature coronary artery disease. The implications are that these fibrin network structures are not lysed easily, raising the possibilities of re-infarctions (Fatah *et al.*, 1996b). The difference in fibrin network structure, as determined with 3-D confocal laser microscope pictures, of healthy persons compared to persons suffering from CHD are indicated in Figure 2.10 (Blombäck *et al.*, 1994b).

In order to prevent the occurrence of myocardial infarction and other arterial thromboembolic events in patients with cardiovascular disease, treatment with acetylsalicylic acid is used (Antiplatelet trialists' collaboration, 1994). Results of a study investigating the influence of the acetylsalicylic acid on the fibrin network structures revealed that a more porous fibrin network structure is formed (Fatah *et al.*, 1996a).

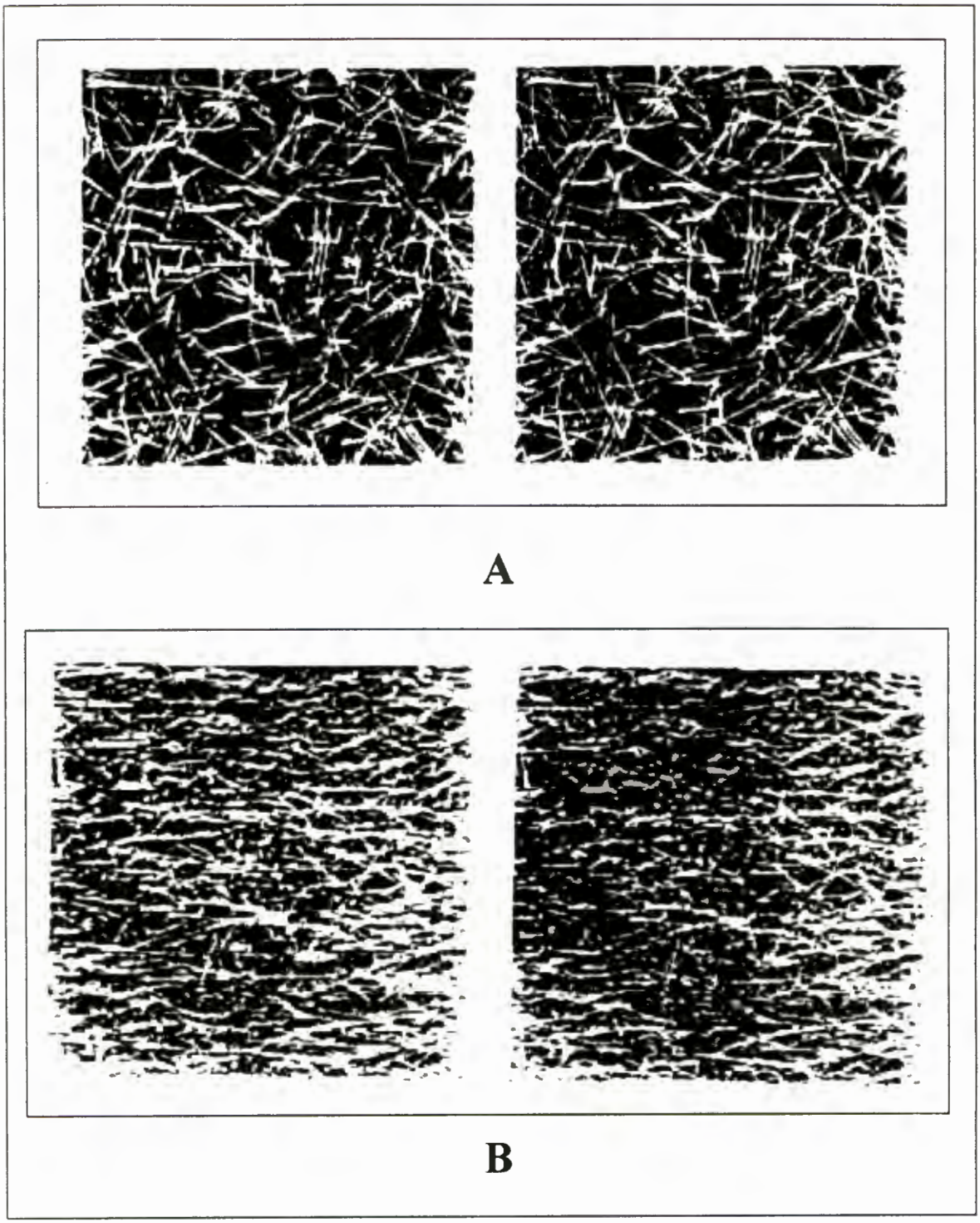

Figure 2.10 **A 3-D confocal laser microscope picture of fibrin gels from a normal individual (A) and a patient with coronary heart disease (CHD) (B) (Blombäck *et al.*, 1994b)**

A follow-up study indicated that low dose acetylsalicylic acid ingestion resulted in increased fibrin network porosity in healthy subjects (Williams *et al.,* 1998). The mechanism is thought to be that acetylsalicylic acid acetylates lysine residues in fibrinogen, as well as in other coagulation molecules, such as prothrombin and antithrombin (Szezeklik *et al.,* 1992). The lysine residues in fibrinogen are important for the cross-linking of fibrin subunits, thereby stabilising the network and increasing its fibrinolytic resistance (Gaffney & Whitaker, 1979). The acetylation of fibrinogen inhibits cross-linking and reduces gel stability. This results in an increase in the flow of fluid through the fibrin network structure, which is important for the perfusion of plasminogen activators into the clot (Blombäck & Okada, 1982) and results in an enhancement of fibrinolysis. The fibre thickness is also important in determining the fibrinolytic efficiency (Carr & Alving, 1995). Thick fibrin fibres are more porous and have an enhanced rate of conversion of plasminogen to plasmin by tissue plasmin activator (t-PA), as a result of an increase in available t-PA binding sites, leading to an increased lysis (Gabriel *et al.,* 1992).

Fibrinolysis is likely to be related to the fibre size in the fibrin network structure. The reason being that coarse fibres are, in contrast to fine fibres, composed of a larger number of protofibrils. Enhanced local concentration of profibrinolytic components in coarse fibres combined with decreased diffusion distances between protofibrils results in relatively faster lysis of these fibres. In the tightest fibrin network structures diffusive flow may limit access of fibrinolytic components to the binding sites on fibres. In the case of the fibrinolysis process, increased concentrations and/or activity of PAI-1 is probably the main cause of impaired fibrinolytic capacity. Increasing fibrinogen concentration decreases porosity of gels in an exponential manner. Fibre numbers are increased but not fibre diameter. The combined effect of these events will decrease the fibrinolytic potential (McDonagh, 1993).

Nair *et al.* (1991b) investigated the effects of glucose on the fibrin networks structure *in vitro*, and examined the fibrin network structure characteristics in the diabetic patient. The results of the study indicated that both the metabolic abnormality and the glucose *in vitro* induced alterations in the fibrin network structure. In the diabetic patient it was found that fibre thickness and permeability are reduced and compaction remains relatively unaltered. As previously noted, the alterations to the fibrin network structure inhibit lysis of the blood clot (Nair *et al.,* 1989). Jörneskog *et al.* (1996) confirmed the results of Nair *et al.*

(1989) when they found an altered fibrin network structure characterised by a reduced permeability coefficient (K_s), while the fibre mass-length ratio (MLR) was not significantly decreased in diabetics. The changes in the fibrin network structure might be due to glycosylation of fibrin when exposed to large quantities of glucose. It has been shown that fibrinogen appears to be only slightly glycosylated in non-diabetic subjects, but to a greater extent in diabetic patients (Lutjens *et al.,* 1985). This reduction in K_s may lead to the decrease in the flow of blood in the circulatory system.

Long-term complications of diabetes are believed to be related to high blood glucose levels (West *et al.*, 1982; Howard-Williams *et al.,* 1984). In addition, cardiovascular disease may be directly related to serum insulin levels (Hillson *et al.,* 1984; Pyorala, 1979).

Abnormalities in the fibrin network structure as seen in the diabetic patients may be related to peripheral vascular disease of both macro- and microangiopathic types. High concentrations and activities of PAI-1 [possibly because of insulin secretion (Vague *et al.*, 1986)] or insulin resistance (Juhan-Vague *et al.*, 1993) have been shown repeatedly in epidemiological and clinical studies to be risk factors for atherosclerosis and thrombosis (Aznar & Estelles, 1994).

Blombäck *et al.*, (1994b) found that not only can abnormal fibrin gel tightness and/or architecture be associated with myocardial infarction at a young age, but acute phase reactions and elevated plasma levels of LDL-cholesterol appear to influence fibrin gel properties. Grossly abnormal fibrin gel architecture appears to be associated with coronary atherosclerotic disease.

Obesity, which is also one of the recognised risk factors for CVD also has an influence on the fibrin network structure. There is agreement that a decreased fibrinolytic activity accompanies obesity. An inverse relationship was found between body-mass index (BMI) and fibrinolytic activity in normal persons (Takada *et al.*, 1993). In obese subjects, PAI activity was shown to be positively correlated with BMI, insulin, and triglyceride (Juhan-Vague *et al.,* 1987). Although most research has concentrated on the fibrinolytic markers, only one study has investigated the formation of fibrin network structures in obese persons (Malan, 1999). This study focused on the fibrin network structure formed in gynoid and

android obese black African women compared to normal weight African women in the *THUSA*-study. The results of this study indicated that the gynoid obese African women formed fibrin network structures more prone to lysis than the android obese women which were still more resistant to lysis than those of the normal weight women. This could possibly be a mechanism through which obesity leads to atherosclerosis and thrombosis.

As discussed in the preceding paragraphs, all CVD risk factors mentioned above have been linked to abnormal fibrin network structures. The consequences of the abnormal fibrin networks may be increases in thrombogenicity and atherosclerosis. This led Shats *et al.* (1997) to investigate the relationship between endothelium cells and fibroblasts with the modified fibrin network structure that is usually formed. The motivation for this investigation was based on indications that the atherosclerotic lesion may be initiated in an interaction of fibrin with the endothelium. Immuno-histochemical studies revealed that the maturing plaque contains layers of fibrin overgrown by the endothelium (Smith & Thompson, 1994). In this study of Shats *et al.* (1997) endothelium cells and fibroblasts were grown on fibrin network structures of either thin or thick fibres developed in plasma with a change in the pH of the plasma. In Figure 2.11 a flow diagram of the modified hypothesis can be seen, indicating the possible role of the fibrin network structure in the formation of atherosclerosis (Shats *et al.*, 1997).

The results indicated that fibrin networks composed of thick fibres induce a more profibrinolytic and antithrombotic environment in endothelium cells and fibroblast cultures than fibrin network composed of thin fibres. Kadish (1979) suggested that in situations of impaired fibrinolysis, fibrin deposits would lead to the exposure of the subendothelium, followed by platelet adhesion, infiltration of lipoproteins, stimulation of smooth muscle cells that together will orchestrate the development of an atherosclerotic plaque. This hypothesis should be modified according to Shats *et al.* (1997) to take into account the different types of fibrin network structures that are formed. The thick fibrin fibres may induce initial disorganisation of endothelial cells. However, as these fibres are more lysable and their interaction with endothelial cells induces a more profibrinolytic/antithrombotic environment, fibrinolysis is promoted and endothelial cells are reorganised. Thin fibres, on the other hand, promote a more antifibrinolytic/prothrombotic environment, and may induce a further exposure of the

subendothelium resulting in increased platelet adhesion, migration of monocytes and growth of the atherosclerotic plaque (Shats *et al.*, 1997).

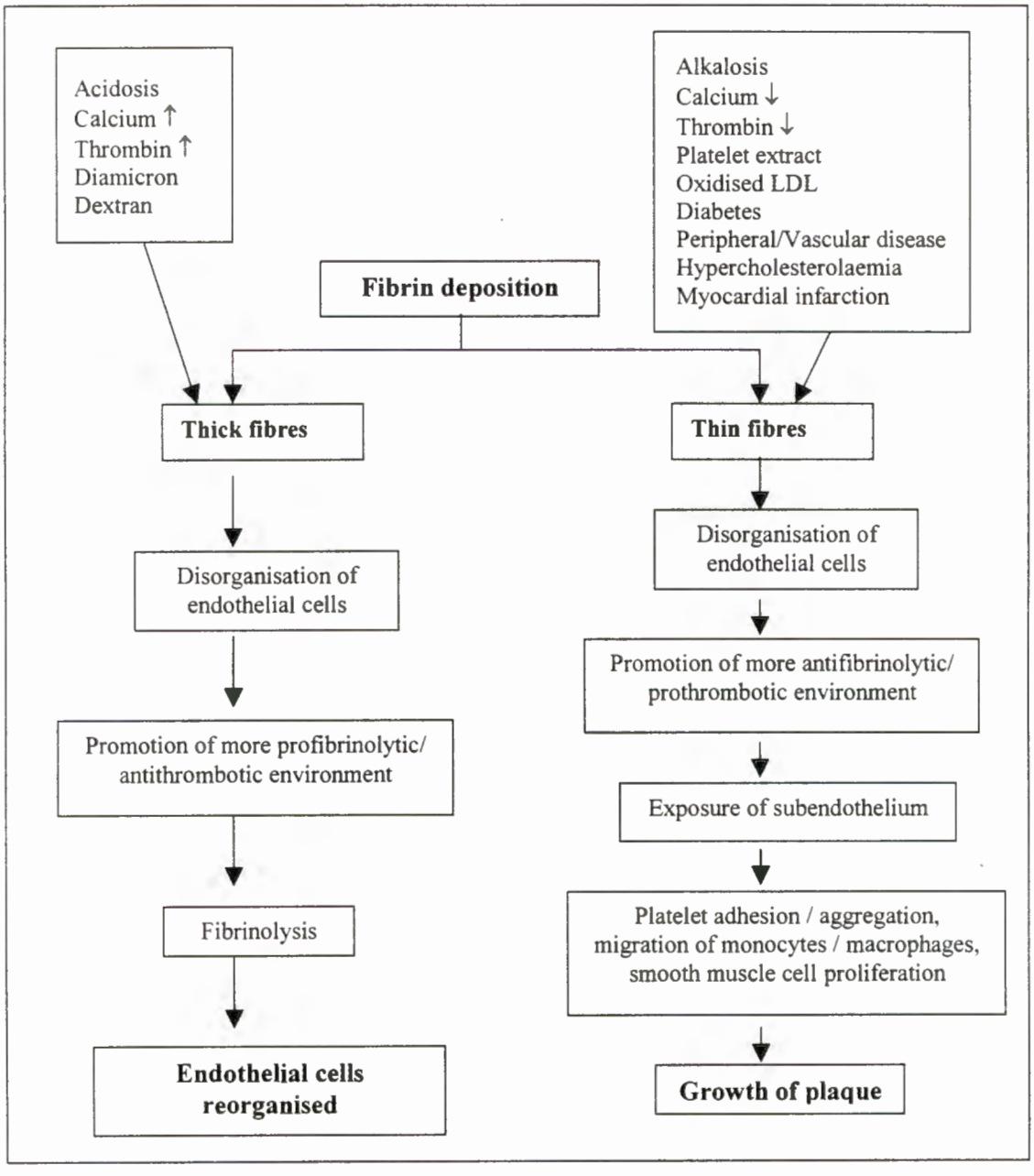

Figure 2.11 A flow diagram of the possible role of fibrin network structure in atherogenesis. The type of fibrin deposited may influence different outcomes. Thick fibres may induce initial disorganisation of endothelial cells. Thin fibres promote a more antifibrinolytic/prothrombotic environment and may induce a further exposure of the subendothelium resulting in increased platelet adhesion, migration of monocytes, and growth of the atherosclerotic plaque (Shats *et al.*, 1997)

2.7 Perspectives for this study

The "accepted" risk factors are linked to atherosclerosis through the damaging effect it has on the endothelial wall of the blood vessels. Although all the mechanisms through which this occurs have not been firmly established, indications are that fibrinogen may play a central role. Fibrinogen is related to most of the "accepted" risk factors, it plays a pivotal role in the haemostatic balance, it determines blood viscosity and is the substrate for the fibrin network. The structure of the network is dependent on various factors, which will determine whether a thin fibrin fibre with high tensile strength or thick fibrin fibres with low tensile strength are formed. The thin fibres are more resistant to lysis than the thick fibres. This abnormality in the architecture of the fibrin network structure is associated with an increased risk of a myocardial infarction at an early age (Fatah *et al.,* 1996b). A recent study performed by Yarnell *et al.* (2000) involving more than 2000 middle-aged men indicated that lifestyle risk factors for IHD may operate partly through changes in haemostatic variables which are risk predictors for cardiovascular events. In order to reduce the risk of CVD it is thus important to modify lifestyle risk factors where possible. Two of the most important modifications to lifestyle are adopting a prudent diet rich in fibre and low in fat, together with an increase in physical activity. In Chapter 3 the influence of physical activity and diet on the haemostatic balance will be described, as this might influence the architecture of the fibrin networks. Currently no data is available on the effect of physical activity on the fibrin network structure.

CHAPTER 3

THE INFLUENCE OF PHYSICAL ACTIVITY AND DIET ON HAEMOSTASIS

3.1 The state of the art

As early as 1910, the inverse association of physical activity and MI was observed, and from then on its existence has been confirmed repeatedly (Willich *et al.*, 1993). Research has shown that physical activity has beneficial effects on multiple physiological variables affecting cardiovascular health (Niebauer & Cook, 1996), and thus a protective effect on the development of CAD, morbidity and mortality (Blair *et al.*, 1995; Francis *et al.*, 1988; Powell *et al.*, 1987). However, mechanisms through which physical activity protects against CHD are not well understood. Connelly *et al.* (1992) and El-Sayed (1996b) both hypothesised that it might be through effects on the haemostatic balance.

There is enough evidence to conclude that some of the protective effects of diet against the development of atherosclerosis, thrombosis and resultant CHD are mediated through effects on haemostasis (Vorster *et al.*, 1997). Changing to a prudent diet that is low in fat, high in fibre, low in salt and rich in antioxidants from fruits and vegetables, with a balance in energy intake to maintain ideal body weight, protects against CVD (Vorster *et al.*, 1997). It is accepted that diet, also through its effects on lipid metabolism and antioxidant properties, plays an important role in the primary and secondary prevention of CVD (Ulbricht & Southgate, 1991).

Investigations on a combination of physical activity and diet interventions have mostly focused on performance enhancement of athletes and recovery after exercise, without considering health outcomes (Leeds *et al.*, 1996; Cogan & Coyle, 1991; Jeukendrup *et al.*, 1997). A diet high in carbohydrate has been recommended for successful participation in

sport (reviewed by Williams, 1997). No conclusive information is available on the influence of these diets on the haemostatic variables. Very little is known about effects on fibrin network structure. This chapter will give a short overview of the available findings with regard to physical activity, diet and a combination of physical activity and diet on haemostatic factors and fibrin network structure.

3.2 The influence of physical activity on haemostasis

3.2.1 Introduction

The effects of exercise on haemostatic function and thus thrombogenic pathways contributing to CVD can be considered in a variety of circumstances in which associations may not always be the same, therefore making generalisations difficult and even impossible. Physical activity may have different metabolic effects according to the degree of previous training, and these effects may be influenced by the intensity of exercise attempted. The recommendation of the ACSM (1998) is that exercise should be dynamic, involving the large muscles for at least 30 minutes or more a session for most days of the week at an intensity of >60% of VO_2 maximum to experience the advantages of activity on health outcomes. Exercise needs to be maintained. It is strongly suggested that the benefit be at least partly mediated through short-term effect on the haemostatic system and its influences on thrombosis being an obvious consideration in this respect. It is also important, as two separate studies (Mittleman *et al.*, 1993; Willich *et al.*, 1993) indicated, that heavy physical exertion in people who are not accustomed to it may increase the risk of myocardial infarction or coronary death. This observation also suggests effects on the haemostatic system, although in this case harmful. The mechanisms that lead to these incidents are not known.

In order to describe the influence of physical activity on haemostasis, this section will firstly deal with the effect of physical activity on fibrinogen during acute activity and then training. This will be followed by the effect of acute and long-term activity on coagulation and fibrinolysis. The effect of dietary changes on the haemostatic balance with special

reference to the glycaemic index will be described. This will finally lead to the research on a combination of physical activity and diet interventions on the haemostatic balance.

3.2.2 The effects of physical activity on fibrinogen

3.2.2.1 The effects of acute physical activity on the plasma fibrinogen levels

El-Sayed and Davies (1995) showed that maximal exercise induced a significant increase in plasma fibrinogen levels. However, when corrected for plasma volume shift, the increase disappeared. Karp and Bell (1974) reported the same results when they found no significant changes in plasma fibrinogen levels in response to moderate or maximal exercise.

Most other reports examining the effect of exercise on plasma fibrinogen levels reported similar results. For example, when post-exercise fibrinogen values were adjusted for plasma volume loss, a multistage maximal treadmill exercise stress test or acute sessions of sub-maximal exercise (Rankinen *et al.,* 1995) did not evoke a significant change in plasma fibrinogen levels in healthy adult individuals. Similarly, plasma fibrinogen levels did not alter significantly after long distance running in highly trained men (Watts, 1991) or exhaustive exercise in untrained individuals (Collen *et al.,* 1977). Likewise, no significant changes in fibrinogen levels were found in response to cycling exercise in young athletes and non-athletes and patients who survived myocardial infarction (Speiser *et al.*, 1988), or during treadmill running in healthy individuals (Hyers *et al.,* 1980). Also, running a half-marathon failed to elicit a significant change in plasma fibrinogen levels (Ponjee *et al.,* 1993).

Although the above mentioned studies have found no change in fibrinogen levels in response to exercise, other investigators have found an increase. For example, Ferguson *et al.* (1979) found a 5% increase in fibrinogen levels after treadmill exercise compared with rest; however, this increase did not reach the assigned level of significance and was within the value reported for plasma volume loss. Ohri *et al.* (1983) employed cycling exercise and found a significant increase in plasma fibrinogen levels by 13% from a mean pre-

exercise value. It should be noted, however, that the rise in plasma fibrinogen was higher than that observed for haematocrit, but lower than that observed for haemoglobin.

Surprisingly, and in contrast to all the previous reports, evidence also exists for decreased plasma fibrinogen levels because of exercise (Bartsch *et al.,* 1990). This study was performed on well-trained athletes competing over a 100 km race. It could be argued that the decrease in fibrinogen levels was probably due to re-hydration with consequence expansion of plasma volume. Research by various groups, especially on the longer endurance items, have shown decreases in the plasma fibrinogen levels (Martin *et al.*, 1985; Østerud *et al.,* 1989). In a recent study performed on 12 well-trained athletes competing in a marathon race, a 25% decrease in fibrinogen was found (Prisco *et al.,* 1998). These decreased values of fibrinogen returned to baseline values after 48 hours of activity. Although the fall in plasma fibrinogen levels after exercise was statistically significant, it is difficult to be considered biologically significant because the magnitude of its reduction seems to be small and within the error of the analytical methods.

Although differences in exercise protocols and methods exist among these studies, it seems unlikely that acute exercise decreases plasma fibrinogen levels because of an enhanced rate of fibrinogen catabolism. This is because of the relative small fibrinogen specificity to plasminogen activators compared with fibrin.

3.2.2.2 The influence of physical training on fibrinogen

When plasma fibrinogen levels of trained and untrained subjects were compared in cross-sectional studies, less contrasting results compared to acute effects of exercise on plasma fibrinogen levels were found. For instance, both the Whitehall (Morris *et al.,* 1990) and the Gothenburg (Rosengren *et al.,* 1990) studies found an inverse relationship between physical activity and fibrinogen. The most comprehensive cross-sectional study is the Arteriosclerosis Risk in Communities study (Folsom *et al.,* 1991). They reported on levels of fibrinogen and factor VII in 5 122 men and 6 506 women aged between 45 and 64 years, and showed that 1 unit of sportive activity reduced the fibrinogen level by 0.3-0.4 g/L. Several other epidemiological studies showed that exercise is negatively associated

with plasma fibrinogen levels, suggesting that regular physical activity may decrease fibrinogen levels.

Some studies have compared individuals before and after training programmes (El-Sayed & Davies, 1995; Ponjee *et al.*, 1993; Suzuki *et al.*, 1992; Stratton *et al.*, 1991). Others have compared athletes and untrained individuals to evaluate the effect of training on plasma fibrinogen levels (Watts, 1991; Connelly *et al.*, 1992; Ernst *et al.*, 1985). One of the most adequately documented trials of exercise and fibrinogen was carried out by Wosornu *et al.*, (1992) on fifty-five men within 12 months of coronary surgery. In a setting of exercise rehabilitation classes the men were randomly allocated to one of three groups. Fifteen patients took part in a programme of aerobic exercise lasting 12 to 60 minutes. The second group of 20 men carried out power exercises involving leg, arm and trunk muscles, also lasting between 12 and 60 minutes. A control group of 20 men received no formal exercise training. Plasma fibrinogen levels were recorded at entry, 3 and 6 months respectively. In both 3 and 6 months, the mean fibrinogen levels in those undergoing aerobic training had fallen significantly, whereas the changes in the power-exercise group and in the control group were inconsistent and not statistically significant.

The diversity of the results reported by various researchers are undoubtedly due to the wide variety in conditioning regimens, study participants, and assay methods for the determination of plasma fibrinogen. Evidence available from longitudinal studies indicated that the individuals most likely to lower their fibrinogen levels in response to physical training are patients with coronary disease and diabetes (Suzuki *et al.*, 1992; Hornsby *et al.*, 1990). The same response can be expected with older individuals (Stratton *et al.*, 1991), but not healthy young (El-Sayed & Davies, 1995; Ponjee *et al.*, 1993) or middle-aged individuals (Rankinen *et al.*, 1994). This could be explained that fibrinogen increases with age. Therefore, young individuals already have low levels of plasma fibrinogen, which will not be decreased by physical training.

The view of El-Sayed (1996b) is that data obtained from the longitudinal studies cited above are often too difficult to interpret because most of these studies did not use a control group. However, the majority of studies that have used a control group have shown no change in plasma fibrinogen levels. In contrast, Meade (1995) concluded that longitudinal studies agree that endurance exercise for several weeks will decrease fibrinogen. The

absolute fall is surprisingly uniform in the region of 0.4 g/L. Exercise may thus be the most practical approach known to date to lower plasma fibrinogen levels. Väisänen *et al.* (1996) found that increased physical activity levels and cardiorespiratory fitness were inversely related to plasma fibrinogen levels in middle-aged men.

MacAuley *et al.* (1996) tested 1 600 subjects between the ages of 16-74 years for physical activity, physical fitness, blood pressure and fibrinogen. The results revealed that an inverse relationship existed between systolic and diastolic blood pressure and physical fitness (VO_2 max). The relationship disappeared when adjusted for confounding factors. This suggests that the protective effect of activity may be independent of physical fitness. Furthermore, the relationship between physical activity, physical fitness and both these risk factors, blood pressure and fibrinogen, appear to have a different pattern, and the benefits of physical activity and physical fitness may act through different pathways.

3.2.3 The influence of physical activity on other coagulation factors

3.2.3.1 Acute activity and the coagulation cascade

In vitro activation of the blood clotting system and increases of the activities of a number of coagulation factors have been reported during and immediately after exercise. Table 3.1 summarises the current literature on the influence of acute physical activity on various haemostatic variables. The apparent discrepancy between younger and older subjects with respect to significant exercise-induced increases of PTF 1+2, TAT and FPA in control groups might be attributed to the presence of atherosclerosis in the older subjects. This agrees with investigations that document an age-related rise in resting levels of PFT 1+2 and FPA, implying that distinct haemostatic alterations are related to the development of vascular disease associated with the normal ageing process (Van den Burg *et al.*, 1995a).

When the effects of acute physical activity on the haemostatic variables are interpreted, FVIIIc and PTF 1+2 indicate constant increases. It is well accepted that exercise causes an activation of blood coagulation. The increase in PTF 1+2 found has coincided with the significant increase in the thrombin-antithrombin complex (Bartsch *et al.*, 1995).

Table 3.1: A summary of changes in some coagulation factors during acute physical activity

Variable	↑	↓	↔	Subjects	Activity	Reference
FVIIIc	*			Sedentary males	Multistage treadmill to exhaustion	Wheeler *et al.*, 1986
	*			Sedentary males	Graded bicycle ergometer to MHR	Van den Burg *et al.*, 1997
	*			Sedentary males	Graded bicycle ergometer to MHR	Van den Burg *et al.*, 1995a
	*			Active males	42km run	Rock *et al.*, 1997
	*			Active males	High intensity treadmill	Chicharro *et al.*, 1995
APTT	*			Active males	Stepwise max. swimming cycling & running	Weiss *et al.*, 1998a
		*		Active males	1h exercise on treadmill at 80% IAT & 100% IAT	Weiss *et al.*, 1998c
		*		Active males	2h triathlon race	Bartsch *et al.*, 1995
		*		Active males	Max. grade cycle ergometer	Van den Burg *et al.*, 1995a
		*		Sedentary males	Graded bicycle ergometer to MHR	Van den Burg *et al.*, 1997
Prothrombin time		*		Sedentary males	Graded bicycle ergometer to MHR	Van den Burg *et al.*, 1995a
		*		Sedentary males	Bruce protocol on treadmill	Ferguson *et. al.*, 1987
			*	Active males	High intensity treadmill	Chicharro *et al.*, 1995
Thrombin time		*		Sedentary males	Bruce protocol on treadmill	Ferguson *et. al.*, 1987
			*	Sedentary males	Exercise	Röcker *et. al.*, 1990
Prothrombin fragment 1+2	*			Endurance athletes	Stepwise max. run, swim, cycle 1h max. run, swim & cycling	Weiss *et al.*, 1998a
	*			Healthy males	1h exercise on treadmill at 80% IAT & 100% IAT	Weiss *et al.*, 1998c
	*			Trained males	42km running speed of 15.35km/h	Prisco *et al.*, 1998
	*			Long distance runners	40 min at 80% VO_2 max. on a treadmill	Kvernmo & Østerud, 1997
	*			Healthy males	2h-Triathlon	Bartsch *et al.*, 1995
	*			Trained males	Bicycle ergometer to MHR	Van den Burg *et al.*, 1995a
			*	CAD patients	1h, 10 min warm & cool down, 20 min aerobic & 20 min ball games	Weiss *et al.*, 1998b
			*	Controls for CAD		
Thrombin-antithrombin complex	*			Endurance athletes	Max run, swim & cycling 1h max run, swim & cycling	Weiss *et al.*, 1998a
	*			CAD patients	1h, 10 min warm & cooldown, 20 min interval & 20 min ball games	Weiss *et al.*, 1998b
	*			Controls for CAD		
	*			Trained males	1h exercise on treadmill at 80% IAT & 100% IAT	Weiss *et al.*, 1998c
	*			Trained males	42km running @ 15.35km/h	Prisco *et al.*, 1998
			*	Sedentary males	40 min@ 80% VO_2 max.-treadmill	Kvernmo & Østerud, 1997
			*	Distance runners	Graded bicycle ergometer to MHR	Van den Burg *et al.*, 1995a
			*	Active controls		
FPA	*			Endurance athletes	Max run, swim & cycling 1h max. run, swim & cycling	Weiss *et al.*, 1998a
			*	CAD patients &	1h, 10 min warm & cooldown, 20 min interval & 20 min ball games	Weiss *et al.*, 1998b
			*	Healthy controls		
	*			Well trained males	1h exercise on treadmill at 80% IAT & 100% IAT	Weiss *et al.*, 1998c
	*			Active males	Marathon race	Röcker *et al.*, 1986
			*	Endurance athletes	2h Triathlon	Bartsch *et al.*, 1995
AT III		*		Active males	Marathon run	Mandalaki *et al.*, 1980
			*	Endurance males	Triathlon	Arai *et al.*, 1990
			*	Active males	100km running	Bartsch *et al.*, 1990
			*	Sedentary males	Exhaustive cycling	Herren *et al.*, 1992
			*	Sedentary males	Maximal treadmill running	De Paz *et al.*, 1992

Table 3.1 (continued)

Variable	↑	↓	↔	Subjects	Activity	Reference
Platelet count	*			Endurance-athletes	Max. run, swim & cycling	Weiss *et al.*, 1998a
					1h max. run, swim & cycling	
	*			Trained males	1h exercise on treadmill at 80% IAT & 100% IAT	Weiss *et al.*, 1998c
	*			H healthy males	85% of MHR of bicycle ergometer	Ohri *et al.*, 1983
FM	*			Endurance athletes	2h triathlon	Bartsch *et al.*, 1995
F IX	*			Sedentary males	Graded bicycle ergometer to MHR	Van den Burg *et al.*, 1995a
	*			Active males	High intensity treadmill	Chicharro *et al.*, 1995
F XII			*	Sedentary males	Graded bicycle ergometer to MHR	Van den Burg *et al.*, 1995a
Fibrinogen			*	Sedentary males	Graded bicycle ergometer to MHR	Van den Burg *et al.*, 1995a
	*			Sedentary males	Graded bicycle ergometer to MHR	Van den Burg *et al.*, 1995b
		*		12 well trained males	42km running with speed of 15.35km/h	Prisco *et al.*, 1998
	*			80 healthy males	85% of MHR of bicycle ergometer	Ohri *et al.*, 1983
		*		Active men/ women	50km cross-country ski race	Østerud *et al.*, 1989
F VII			*	Sedentary males	Graded bicycle ergometer to MHR	Van den Burg *et al.*, 1995a
		*		Active men/ women	50km cross-country ski race	Østerud *et al.*, 1989
		*		Active males	4.8km run at near max. speed	Hansen *et al.*, 1990b

FVIIIc= Factor VIII, APTT = activated partial thromboplastin time, FPA = fibrinopeptide A, AT III = antithrombinIII, FM = fibrin monomer, FIX = Factor IX, FXII = Factor XII, FVII = Factor VII, IAT = individual anaerobic threshold, MHR = maximal heart rate, VO_2 max.= maximal oxygen uptake

Several of the variables showed conflicting results, which makes it impossible to draw a definite conclusion. Blood is hyper-coagulable when it is checked immediately after strenuous exercise, and this has been attributed to an increase in Factor VIII coagulant activity (Ferguson *et al.*, 1987; Hansen *et al.*, 1990b; El-Sayed *et al.*, 1996). With the increase and PTF 1+2, the activation of prothrombin to thrombin is suggested. Thus strenuous physical activity induces significant thrombin generation and thrombin activity, changes that could well be relevant when considering the increased risk of ischaemic heart disease that follows unaccustomed physical activity, particularly if there is an inadequate increase in fibrinolytic activity.

3.2.3.2 The effect of training on blood coagulation

Very few published results seem to be available regarding the influence of exercise training on the blood coagulation mechanism. Ferguson *et al.* (1987) studied 60 healthy males in three physical fitness categories: sedentary, joggers, and marathon runners. No differences between groups were found in prothrombin time and APTT at rest or after a maximal treadmill test. Watts (1991), who compared a group of athletes with a non-exercising control group, reported similar results. There were no significant differences between the two groups in thrombin time at rest.

Prolongation of APTT (hypocoagulability) after a training programme was found in post myocardial patients, suggesting that physical training resulted in the suppression of coagulability (Suzuki *et al.*, 1992). El-Sayed *et al.* (1996) confirmed earlier reports and showed no significant effects of conditioning on prothrombin time, APTT and thrombin time at rest or in response to exercise.

Ponjee *et al.* (1996) evaluated the effects of 9 months of physical training with increasing intensity on selected blood coagulation parameters in sedentary individuals. Compared with pre-training, Factor VIII (FVIII) activity and antigen levels did not change significantly after the training programme. This agrees with a cross-sectional study that showed no change in resting levels of FVIII activity and antigen in endurance-trained athletes compared with a control group. Similarly, physical conditioning programs did not alter FVIII activity and antigen level at rest (Rankinen *et al.*, 1994; Boman *et al.*, 1994) or in response to exhaustive cycling exercise (El-Sayed *et al.*, 1996). DeSouza *et al.* (1998) recently indicated that the plasma fibrinogen levels in physical active postmenopausal women increased by 50% less with age than in sedentary postmenopausal women. This would appear to be associated with a reduction in CVD risk for physically active postmenopausal women.

It is not possible to come to any definite conclusions on the effect of training on the haemostatic balance as different protocols are followed in the various studies. It seems that training leads to a reduction in fibrinogen levels in the elderly, but not in young healthy subjects. Further research in this area is needed.

3.2.4. The influence of physical activity on fibrinolysis in the haemostatic balance

3.2.4.1 Acute activity and fibrinolysis

Numerous studies have demonstrated that acute exercise rapidly increases fibrinolytic activity. Most of the studies have been small. Because the protocols of studies differed, individual studies will be reviewed separately in this section.

The fact that exercise enhances blood fibrinolytic activity was first observed by Hunter in 1794 and later confirmed by Biggs *et al.* (1947). In the last few years the effects of exercise on blood fibrinolysis have been studied extensively. A summary of effects of different markers of fibrinolysis is given in Table 3.2. There is agreement that a bout of intense exercise induces a significant activation of fibrinolysis, mediated by the release of t-PA from the vascular endothelial cells (El-Sayed *et al.,* 1996). Several investigators employed a global measure such as euglobin clot lysis assay or the fibrin plate lysis technique to assess overall fibrinolytic activity in response to physical exercise. Although the euglobulin clot lysis method and the fibrin plate lysis technique provide good overall assessments of the fibrinolytic system, they are of poor predictive value with respect to identification and quantification of the specific changes that occur in the individual components of this system.

Exercise enhanced fibrinolytic activity was manifested by 20 to 80% shortening in euglobin lysis time (Ferguson *et al.,* 1987; Bartsch *et al.*, 1990). Marked increases in fibrinolytic activity do not become apparent until 50% of maximal heart rate is attained (Andrew *et al.,* 1986). The largest increase occurs between 70 and 90% of maximal workload (Davis *et al.,* 1976; Andrew *et al.,* 1986). This hyperfibrinolysis is transient and returns to the baseline level after 45 to 60 minutes of intense exercise (Ferguson *et al.,* 1987), or two hours after long-distance running.

Table 3.2: A summary of changes in fibrinolytic factors during acute physical activity

Variable	↑	↓	↔	Subjects	Activity	Reference
Euglobulin lysis time		*		Triathletes	Triathlon	Bartsch *et al.*, 1995
	*			CHD males		
	*			Sedentary males	Bruce protocol	Ferguson *et al.*, 1987
	*			Trained males	42km run @ 15.25km/h	Prisco *et al.*, 1998
t-PA	*			Triathletes	Triathlon	Bartsch *et al.*, 1995
	*			Sedentary males	Max. bicycle ergometer	Van den Burg *et al.*, 1995a
	*			Sedentary males	Max. bicycle ergometer	Van den Burg *et al.*, 1997
	*			Athletes	Max treadmill	Kvernmo & Østerud, 1997
	*			Active males	Running	Hansen *et al.*, 1990b
PAI-1		*		Sedentary males	Max. bicycle ergometer	Van den Burg *et al.*, 1995a
		*		Trained males	42km run @ 15.25km/h	Prisco *et al.*, 1998
			*	Healthy males	1h, 40 aerobic training, 20 min warm up & cooldown	Weiss *et al.*, 1998b
FbDP	*			Sedentary males	Bruce protocol	Ferguson *et al.*, 1987
	*			Sedentary males	Max. bicycle ergometer	De Scalzi *et al.*, 1987
	*			Active males	Max. bicycle ergometer	De Scalzi *et al.*, 1987
	*			Trained males	42km run @ 15.25km/h	Prisco *et al.*, 1998
D-dimer	*			Sedentary males	Max. bicycle ergometer	Van den Burg *et al.*, 1995a
	*			Trained males	42km run @ 15.25km/h	Prisco *et al.*, 1998
	*			CAD patients	1h, 40 aerobic training, 20 min warm up & cooldown	Weiss *et al.*, 1998b
	*			Healthy males		
Plasmin anti-plasmin complex	*			Triathletes	Triathlon	Bartsch *et al.*, 1995
	*			Sedentary males	Max. bicycle ergometer	Van den Burg *et al.*, 1995a
	*			CAD patients	1h, 40 aerobic training, 20 min warm up & cooldown	Weiss *et al.*, 1998b
	*			Healthy males		
Plasminogen			*	Triathletes	Triathlon	Bartsch *et al.*, 1995
			*	Sedentary males	Max bicycle ergometer	Collen *et al.*, 1977
α2-antiplasmin			*	Triathletes	Triathlon	Bartsch *et al.*, 1995

t-PA = tissue plasminogen activator, PAI-1 = plasminogen activator inhibitor 1, FbDP = fibrin degradation product; CAD = coronary artery disease

The mechanism responsible for exercise-induced hyperfibrinolysis is not entirely understood and the biological significance of such a response is to some extent questionable. Adrenoreceptor stimulation was suggested as a possible pathway for the release of plasminogen activator (El-Sayed & Davies, 1989), since β-blocking with propranolol partially decreased, but did not completely abolish, the normal fibrinolytic response to vigorous exercise (El-Sayed, 1992). In addition, the exercise-induced t-PA release occurs before adrenaline (epinephrine) increases during exercise. These findings may suggest that the main release of t-PA in exercise is mediated by some other nonadrenergic mechanism, possibly vasopressin (El-Sayed, 1990).

Blood fibrinolysis is regulated not only by t-PA but also by PAI-1. Fibrinolytic activity increases after exercise because of an increase in t-PA (Rankinen *et al.,* 1995; Szymaski & Pate, 1994). This increase is associated with the intensity of the exercise as well as the training status of the individual. High and low resistance exercise have an increased effect on t-PA (El-Sayed, 1993). The response of PAI-1 is also related to the intensity of the activity.

The significance of increased *in vitro* fibrinolytic activity following exercise is not fully understood. Numerous investigators have attempted to relate the *in vitro* activation of fibrinolysis with changes in fibrinogen level. *In vivo* alterations in markers pertinent to fibrinogen and/or fibrin degradation products (Fb/FgDP) provided valuable information pertinent to blood haemostasis in exercise and training. Plasma Fb/FgDP responses appear to be related to the intensity of exercise and the training status of the individual. In light of this evidence, it can be assumed that vigorous exercise results in hyperfibrinolysis *in vivo*, as manifested by an enhanced splitting of fibrin and/or fibrinogen. Other studies, however, reported no change in Fb/FgDP in response to exercise. Karp & Bell (1974) failed to demonstrate changes in Fb/FgDP in patients and in healthy individuals after moderate and maximal exercise protocols, respectively. Similar findings were also reported after 5 minutes of strenuous cycle exercise (Marsh & Gaffney, 1982) and in response to a graded supine cycling exercise test (Bounnameaux *et al.*, 1992). Therefore, the actual effect of exercise on Fb/FgDP is debatable.

A study on the response of healthy males to moderate and very heavy exercise, found a marked activation of the fibrinolytic system after moderate exercise as indicated by t-PA antigen levels and PAP concentrations rising on the average three and two-fold, respectively. The FbDP only increased by 20% and reached statistical significance in the recovery phase only. In response to very heavy exercise, t-PA antigen and PAP rose five and three-fold respectively, while concentrations of FbDP increased significantly by 50% during recovery (Weiss *et al.*, 1998a).

3.2.4.2 The effect of training on fibrinolysis

Although the mechanisms by which physical exercise reduces CVD are as yet unclear, it is possible that exercise seems to be influenced by the training status of the individual.

Previous studies showed a higher t-PA release and reduced formation of t-PA/PAI-1 complexes in trained compared with untrained individuals (De Paz *et al.*, 1992). This concurs with the evidence reported by Szymanski *et al.* (1994), who compared the effect of maximal treadmill exercise with fibrinolytic activity in physically active and inactive men. These authors demonstrated higher post-exercise values of t-PA in active individuals. However, Ferguson *et al.* (1987), using euglobulin lysis time, demonstrated that marathon runners had similar levels of resting fibrinolytic activity to that of less active individuals. But, the runners had greater fibrinolytic response to maximum treadmill exercise (76% compared to 63% for joggers and 55% for sedentary individuals).

Two earlier studies (Menon *et al.*, 1967; Moxley *et al.*, 1970) demonstrated a significant increase in blood fibrinolysis at rest in physically active individuals compared with a sedentary control group. Szymanski *et al.* (1994), using specific techniques for measuring t-PA, showed that highly active and regularly active men had slightly higher resting t-PA activity than inactive men. In addition, a significantly higher post-exercise t-PA level was noted in the active groups compared with the inactive group. PAI-1 activity, on the other hand, was highest in the inactive group and lower in the highly active group at rest. Results also showed that the reduction in PAI-1 activity in response to exercise is positively related to the training level of the individual. It was suggested that physical conditioning enhanced blood fibrinolytic response to exercise but not at rest. Higher t-PA activity at rest, and in response to exhaustive treadmill exercise, was observed in middle-distance runners compared to inactive individuals (De Paz *et al.*, 1992). Furthermore, PAI-1 activity and antigen were higher in the sedentary group than in the active group at rest.

Speiser *et al.* (1988) reported lower PAI-1 values in young athletes than in age-matched sedentary individuals, and elderly sportsmen had lower PAI-1 values compared with patients with myocardial ischaemia. The physically active individuals also had a greater increase in t-PA activity in response to exercise than the inactive individuals.

Stratton *et al.* (1991) studied fibrinolytic parameters in a group of young and elderly healthy individuals before and after a 6-month training period. Although no significant changes occurred in the young, the resting values in the elderly individuals showed a 39% increase in t-PA activity, and a 58% decrease in PAI-1 activity. Williams *et al.* (1980) demonstrated a shorter euglobulin lysis time and enhanced fibrinolytic activity at rest and in response to venous occlusion after a 10-week endurance training period. Similar results were reported by Boman *et al.* (1994) who assessed the effect of a 14-day skiing tour on fibrinolytic activity. After the first week of the skiing tour there were significant drops in PAI-1 activities and t-PA antigen levels, but t-PA activities were unchanged.

In contrast, El-Sayed *et al.* (1996) failed to show any changes in overall fibrinolytic activity (as assessed with the fibrin plate method), and specifically in t-PA after 12 weeks of endurance training in young individuals either at rest or in response to maximal cycling tests. These findings concur with the evidence reported by Rankinen *et al.* (1994). They showed that a combination regimen of aerobic threshold training and a fat-modified diet had no significant effects on t-PA and PAI-1 antigen levels. Evidence also suggests that physical training is associated with a reduction in PAI-1 activity, and cigarette smoking cessation potentiates this reduction (Gris *et al.,* 1990). Interestingly, detraining was associated with a rebound (increase) of PAI-1 activity to the pre-training level. De Geus *et al.* (1992) showed a decrease in plasminogen inhibitor activity after the initial 4 months of an endurance-training programme with a further decrease after the following 4 months. However, the decrease in PAI-1 activity failed to reach the assigned level of significance due to the large variance within groups and seasonal variations.

The influence of regular physical exercise on t-PA and PAI-1 was determined in patients after a myocardial infarction (Estelles *et al.,* 1989). The results showed that by the sixth month of training, fibrinolytic activity measured by t-PA capacity had decreased significantly in the patients who were not participating in the rehabilitation programme. It had increased slightly in the patients involved in the rehabilitation programme compared with the initial values. It was also observed that while PAI-1 activity remained constant or decreased slightly in patients after 6 months in the rehabilitative sports programme, these PAI-1 levels increased significantly in patients who were not in the programme. Similar results have been observed by Suzuki *et al.* (1992) who found a significant reduction in PAI-1 after 1 month of systematic physical training in 56 post-myocardial infarction

patients. A more recent study performed by Páramo *et al.* (1998) on the effect of a cardiac rehabilitation programme supports the above results. Marked decreases were found in PAI-1 after three and nine months of rehabilitation compared to baseline values.

The elevated t-PA activity and depressed PAI-1 activity in physically active individuals under basal conditions may partly explain the protective role of physical training against the development of vascular disease. The exact effects of physical training on the haemostatic balance needs further research. At this stage is seems that regular physical training favours the fibrinolytic system. This means that the activation of fibrinolysis occurs at a lower level of exercise intensity than activation of coagulation. In response to very heavy exercise, the fibrinolytic system seems to be activated to a higher degree than coagulation, suggesting that fibrinolytic activation counteracts exercise-induced thrombin and fibrin formation (Weiss *et al.,* 1998a).

3.3 The effect of diet on haemostasis

3.3.1 Introduction

The notion that diet influences haemostasis is not new. But the relationship of the total diet with the different variables of haemostasis are far from clear (Vorster *et al.,* 1997). When energy restricted diets resulted in weight loss, the haemostatic profile improved because of decreases in blood viscosity (Poggi *et al.*, 1994), factor VIIc (Slabber *et al.,* 1992) and PAI-1 concentration and activity (Huisveld *et al.,* 1990). An increase in total fat intake increased factor VIIc (Mennen *et al.*, 1996). In addition to the effect of energy and fat, intakes of carbohydrates, fibre, micronutrients such as vitamins A and E and several foods such as onions, garlic, green tea and peppers are related to changes in the haemostatic system (Vorster *et al.,* 1997).

The prudent diet, recommended to lower risk of developing chronic diseases of lifestyle, is high in carbohydrate and fibre and low in fat, especially saturated fatty acids. Carbohydrates can be classified as simple (mono- and disaccharides sugars), complex (polysaccharides such as starch), and non-starch polysaccharides (fibre). Simple

carbohydrates are absorbed rapidly and cause large rises in blood glucose after ingestion. Complex carbohydrates especially in an unrefined form containing fibre, are absorbed more slowly and produce flat glucose responses (Coyle & Coyle, 1993; Jenkins *et al.*, 1986).

As mentioned earlier, this difference in glucose response, because of effects on glucose and insulin levels is proposed to influence haemostatic variables and the fibrin network structure. In this study a high and low glycaemic index meal was used to investigate the effect that different carbohydrate meals may have on haemostatic variables and the fibrin network characteristics. A short overview of the GI will be given in this section

3.3.2 The glycaemic index (GI)

Recognising the unpredictability of blood glucose response to various CHO-rich foods, Canadian nutritionists introduced the concept of the glycaemic index (GI) in the early 1980's (Jenkins *et al.*, 1981). The GI is a ranking of foods based on their actual postprandial blood glucose response compared to a reference food, either glucose or white bread. The clinical utility of the GI has been demonstrated in a number of studies. Manipulating food choice to lower the GI of the diet improves glucose control in diabetics (Brand *et al.*, 1991; Collier *et al.*, 1988) and reduces hyperlipidaemias (Jenkins *et al.*, 1985).

The GI is calculated by measuring the incremental area under the blood glucose response curve following ingestion to two hours of a test food providing 50g of CHO compared with the area under the blood glucose curve following an equal CHO intake from the reference food. All tests are conducted after an overnight fast (Burke *et al.*, 1998).

$$\text{GI \%} = \frac{\text{Blood glucose area after 50g CHO in test food}}{\text{Blood glucose area after 50g CHO in reference food}} \times 100$$

By using the GI, or comparison to a reference food, the problem of considerable inter-individual variability in the absolute glycaemic response to foods is countered. In essence, the GI reflects the rate of digestion, absorption and immediate metabolism of a CHO-rich food. The intermediate metabolism results in glucose being absorbed from the portal blood after carbohydrates have been absorbed from the digestive track. The tempo of glucose conversion to glycogen, is insulin-dependent. The limitation of the GI is that it only gives a value for a single food and not meals. Wolever and Jenkins (1986) have shown that the GI for individual food can be used to predict the GI of a mixed meal. The following factors influence the digestion, absorption and intermediate metabolic rate and therefore the GI of CHO-rich foods.

- Food form, including particle size due to degree of milling or processing, the presence of intact grains, and texture and viscosity including the presence of soluble fibre.
- Degree of food processing and cooking, which influences the degree of gelatinisation or retrograding of starch and disruption to the cell structure. Uncooked starch found in greenish bananas have a low GI; Starches eaten cold have a low GI because of retrogradation of starch.
- The presence of fructose or lactose (both have lower GI)
- The ratio of amylopectin and amylose in starch (the amylose form of starch has a slower rate of digestion due to its linear structure)
- Starch-protein or starch-fat interactions
- The presence of anti-nutrients such as phytates and lectins. Antinutrients contribute to the low GI of legumes.

3.3.3 The applications of the GI concept

The GI of foods is used to assist in choices of CHO containing foods for better metabolic control in diabetic patients (Brand *et al.*, 1991; Fontvieille *et al.*, 1992) and enhanced endurance performance in athletes (Walton & Rhodes, 1997). A low GI diet has beneficial effects on non-insulin dependent diabetes (Wolever *et al.*, 1992) and on insulin-dependent diabetes (Fontvieille *et al.*, 1992) on a long-term basis. The improvement of blood glucose

control and plasma lipid levels may be of major importance in the prevention of micro- and macro-angiopathy (Ducimetiére *et al.*, 1980).

The implementation of the GI in sport performance is based on the fact that the body uses carbohydrate as fuel during activity and stores it in the form of glycogen in the muscle and liver. The carbohydrate stores are small and can produce energy for only two to three hours. After this the stores are depleted and blood glucose levels start to decline. This may result in disorientation and possibly diminished performance (Leeds *et al.*, 1996). The ingestion of carbohydrates 30 to 60 minutes prior to prolonged exercise results in increased muscle glycogen utilisation during exercise and decreases the time to fatigue in exhaustive exercise. These negative outcomes might be due to the elevated blood glucose and to reactive high insulin levels prior to exercise. Thomas *et al.* (1991) has indicated that these negative effects can be avoided by consuming a low GI food. Taking a low GI-meal would result in a slow release of glucose, to promote sustained carbohydrate availability and less variations in insulin levels. The effects of the glycaemic index of a food on the haemostatic balance during exercise are not known.

3.3.4 Research on GI, physical activity and haemostasis

Studies on the influence of the GI of a food on sport performance and control diabetes have been performed, but the subsequent influence on the haemostatic balance has not received attention.

Wee *et al.* (1999) examined the influence of high and low GI meals on endurance running capacity. These authors showed that there was a relative shift in substrate utilisation from CHO to fat when a low GI meal had been ingested before exercise compared with a high GI meal suggesting that the amount of rate of CHO "delivered" for metabolism was not sufficient. There were no differences in running capacity in their trial. These results indicated that if the GI of the pre-exercise meal is used to manipulate substrate utilisation during exercise, careful consideration should be given to the amount and type of CHO, as well as the time of the meal before exercise commences. Clearly, more research is needed in this area.

The only study that could be found that investigated the influence of diet intervention on the fibrin network structure was performed by Veldman *et al.* (1997). In this study pectin supplements were given to a group of hyperlipidaemic patients. The results indicated that pectin had a beneficial effect on both the lipid metabolism and haemostasis. The fibrin network structures formed after the supplementation of pectin were more permeable with lower tensile strengths. No changes occurred in the fibrinogen concentrations. In a follow-up study Veldman *et al.* (1999) hypothesised that the mechanism might be mediated by the acetate that is formed when pectin is fermented in the gastro-intestinal tract. In vitro experiments showed that acetate influenced the formation of the fibrin network (Veldman *et al.*, 1999). This study opened the possibility that dietary interventions may influence fibrin formation.

The objective of this study was to determine the influence physical activity has on the fibrin network structure, as information is only available on effects on other haemostatic variables. Moreover, results are contradictory and inconclusive. Secondly, the effect of a GI meal on the haemostatic variables and fibrin network structure was investigated, as there is no data available regarding this effect. Finally, a combination of a GI meal with physical activity was determined in order to know what the effects of the GI of the pre-exercise meal are on metabolic and haemostatic variables during and after exercise of sportsmen. The results of this study should give some insight in the underlying mechanisms through which physical activity and dietary changes reduce CVD risk factors.

CHAPTER 4

METHODS

4.1 Introduction

This study formed part of a Sports Nutrition intervention study at the Potchefstroom University for Christian Higher Education. The aim of this study was to determine the influence of physical activity on the fibrin network structure of sedentary and active male subjects. Various other metabolic parameters were also determined in order to explain the changes in fibrin network structure. The results of this study will be presented in the format of two scientific manuscripts in Chapter 5. A pre-exercise meal intervention was given to the same subjects, to determine whether different glycaemic index pre-exercise meals will have different influences on the metabolic parameters.

4.2 Study design

A case-control study with a randomised intervention of a high and low glycaemic index pre-exercise meal was done. A group of highly trained males was recruited on a basis of availability. A sedentary group that were age and sex-matched, was also recruited. All subjects were in good health: entry criteria included no history of angina, myocardial infarction, stroke, chronic pulmonary disease, diabetes, hypertension, exercise-limiting orthopaedic impairment, psychiatric illness, or sleep disorders. The inclusion and exclusion criteria are shown in Table 4.1. Each of these groups of subjects was taken through the protocol as illustrated in Figure 4.1.

Tabel 4.1: The inclusion and exclusion criteria for the recruitment of active and sedentary subjects

Inclusion criteria:	
•	Ages between 18 to 30 years
•	BMI of 18 – 25 kg/m^2
•	Active subjects: > 30 km per week training
•	Best 10 km time < 40 minutes
•	Sedentary subjects no training
Exclusion criteria:	
•	Chronic disease
•	Smoking
•	Alcohol consumption
•	Medication
•	Supplementation

The study design determined that the active and sedentary males would be 12h-fasted and subjected to a maximal exercise. Blood samples were taken at fasting, maximal exercise and 30 minutes of recovery. The same subjects were then recruited to participate in a randomised high and low GI meal intervention. The subjects were fasted when blood samples were drawn. A high or low GI meal was then given. One hour after ingestion of the meal, blood samples were taken once more. The males were then subjected to a maximal exercise after which blood was drawn again with a final sample taken at 30 minutes of recovery. The various parameters were measured at time 1 through time 11.

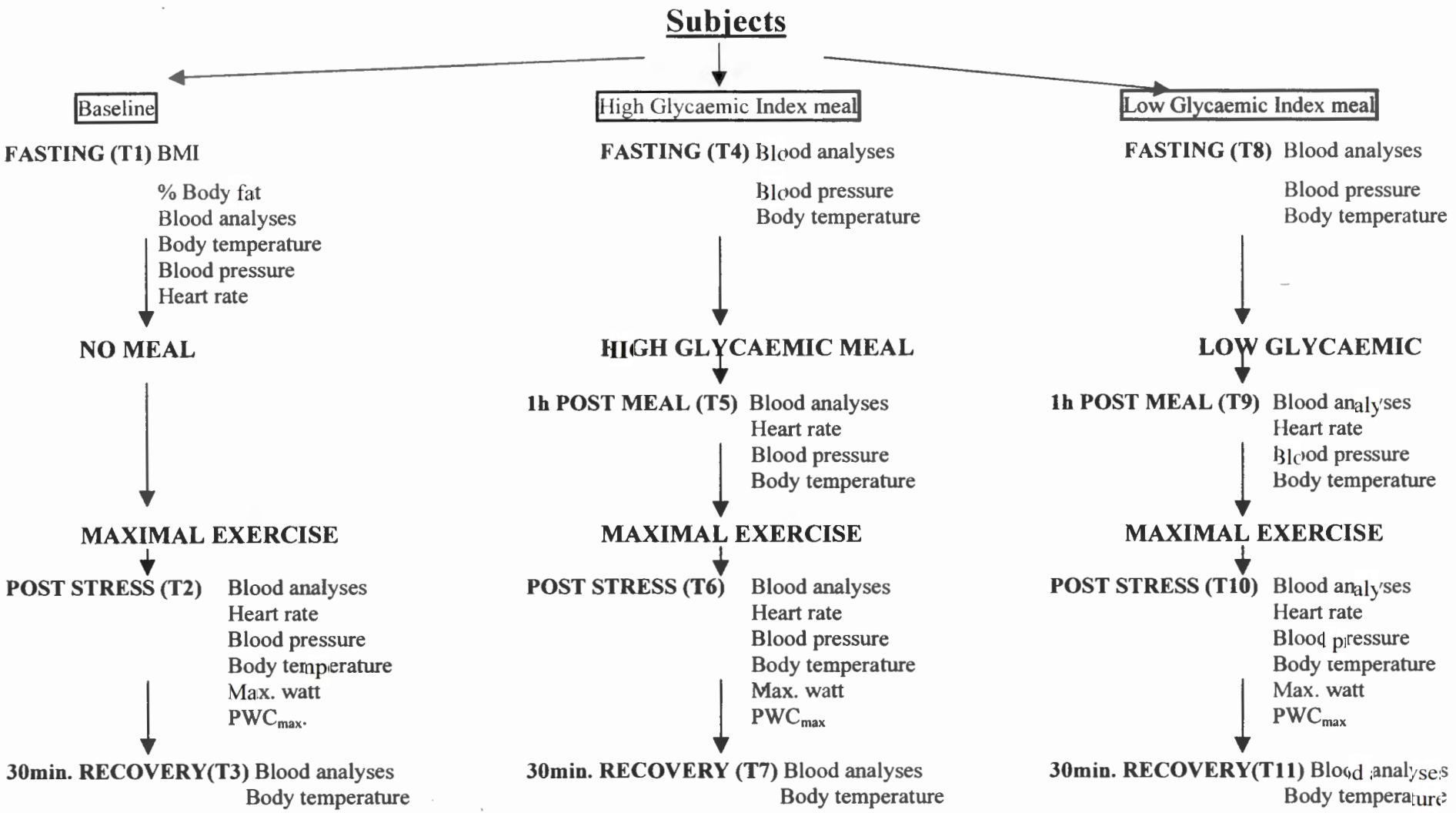

Figure 4.1 **A schematic representation of the study design together with the parameters measured at each time interval.**
Max. watt = maximal resistance in watt; PWC_{max} = physical work capacity at maximal watt

4.3 Subjects

The highly active group consisted of 15 healthy, highly exercised males between the ages of 18 to 30 years. These males ran at least 30 km per week and had had a best 10 km time of less than 40 minutes during the previous three months. The body mass index (BMI) of these males was between 18 and 25 kg/m^2. To determine whether the response of a maximal activity on the fibrin network structure and haemostatic characteristics in active males differ from that of sedentary males, a group of 15 sedentary males was recruited. They were matched with the active group regarding sex and age, and attempts were made to match also for BMI. The sedentary males had not participated in any organised aerobic activity during the previous 6 months.

All subjects abstained from any alcohol, smoking, use of dietary supplements and medication for 10 days before testing. The subjects did not participate in any physical activity for 24 hours preceeding the testing. After the protocol was explained to the participants, each subject signed an informed consent form (Addendum A). The study was approved by the Ethics Committee of the PU for CHE (no. HHK4M5-95).

4.4 Procedures and apparatus utilised for data collection

4.4.1 Risk screening questionnaire

To ensure that inclusion and exclusion criteria were met and no CVD risk factors were present, a risk screening questionnaire (Addendum B) was completed for each subject.

4.4.2 Demographic information recorded during baseline measurements

4.4.2.1 Stature

Stature was measured with the use of a *Seca®* stadiometer (Germany). The stretch stature method was used in determining the height of all the subjects as described by Norton and

Olds (1996). This method required the subjects to stand with their feet together and the following anatomical parts touching the scale: the heels, buttocks and upper part of the back. The head was placed in the Frankfort plane, which meant that it would not necessarily touch the scale. The Frankfort plane was achieved when the orbitale (lower edge of the eye socket) was in the same horizontal plane as the tragion (the notch superior to the tragus of the ear). When aligned, the vertex is the highest point on the skull.

The measurer placed the hands along the jaw of the subject with the fingers reaching to the mastoid processes. The subject was then instructed to take and hold a deep breath while at the same time keeping the head in the Frankfort plane. The recorder then gently applied an upward lift through the mastoid processes. The headboard was firmly placed on the vertex, crushing the hair as much as possible. The measurement was taken at the end of a deep inward breath. The height was recorded to the nearest 0.5 cm.

4.4.2.2 Body mass

The body mass of the subjects was recorded with a calibrated *Seca®* beam balance (Germany) to the nearest 100g. The subjects were clothed in a light shirt and light-weight shorts and bare feet. They were then required to stand on the scale with their weight evenly distributed between both feet. The head was up with the eyes looking directly forward (Norton & Olds, 1996). The body mass was recorded when the balance beam stabilized.

4.4.2.3 Body Mass Index (BMI)

The Body Mass Index (BMI), or the Quetelet Index (ACSM, 1995), was used to assess weight relative to height. It was then calculated by the following equation:

$$\textbf{BMI (kg/m}^2\textbf{)} = \frac{\textbf{Body Mass (kg)}}{\textbf{Height (m)}^2}$$

BMI is a relative good indicator of total body composition in populations-based studies and is related to health outcomes. According to Jáequier (1987) the desirable BMI range for adult men and women is between 20 and 24 kg/m^2.

4.4.2.4 Percentage body fat

The percentage body fat was determined by means of a *Slimguide®* skinfold caliper measuring to the nearest 0.5 mm. The principle behind this technique is that the amount of subcutaneous fat is proportional to the total amount of body fat.

Various regression equations have been developed to predict body density from skinfold measurements. The skinfolds which were measured during this study included the triceps, sub-scapular, supra-spinale, abdominal, frontal thigh and medial calf. These measurements were taken as described in Norton and Olds (1996). For the determination of the percentage body fat, the sum of the skinfolds measured were totalled and used in the following calculation (McArdle *et al.*, 1994):

$$\textbf{Percentage body fat (\%)} = \Sigma\ \textbf{6 skinfolds} \times \textbf{0.1051} + \textbf{2.585}$$

4.4.2.5 Clinical examinations

A qualified nursing sister performed the clinical examinations. The subjects were examined for any infections and oral temperatures (SMIC, China) were taken. The resting systolic and diastolic blood pressures were measured with a sphygmomanometer (Tycos®, USA) recording the values at the first and the fifth Korotkoff sound. A polar heart rate monitor (Cardio Sport®), placed on the subjects before the exercise intervention, recorded the resting heart rate.

4.4.3 Exercise intervention and the calculation of physical work capacity

4.4.3.1 Exercise intervention

The exercise intervention was implemented to determine the effect of a maximal exercise activity on the various biochemical measurements of the haemostatic balance of the sedentary and active males. The subjects were tested between 6:30 and 10:00 am after they were fasted for 12 hours. No stressful activity was to be performed during the 24 hours before testing. The percentage body fat was determined as described in section *4.4.1.4.* The height (stature) and mass of each subject was determined as described in section *4.4.1.1* and *4.4.1.2.* The body temperature, resting heart rate and resting blood pressure was recorded as described in section *4.4.1.5.* All the measurements were recorded on the data sheets (Addendum C). Fasting blood samples were drawn as described in section 4.4.4. The subjects then started with the maximal activity on the bicycle ergometer.

A scalar exercise test was performed on a bicycle ergometer (Monark™) inside an air-conditioned laboratory with the temperature maintained at 23°C. The initial workload was 50 W which was increased, with the sedentary subjects, by the same amount every 4 minutes. The resistance for the active group was increased with 100 W increments every 4 minutes (De Scalzi *et al.,* 1987). Heart rate (*Cardio Sport*®) and blood pressure (sphygmomanometer) were recorded at rest and before each increase in workload. The rate of perceived exertion (RPE) was recorded with the use of the 0 to 10 RPE-scale together with the heart rate and blood pressure recordings before the work load was increased (Noble *et al.,* 1983). The test was terminated when the subjects attained the maximal heart rate predicted for their age (220 – age) as described by Karvonen formula in the ACSM's guidelines (1995). All the results were reported on the data sheet (Addendum C).

Within 5 minutes after maximal activity another blood sample was drawn. The subjects were then asked to remain seated on an upright chair for the following 30 minutes, after which the recovery sample was drawn in the same manner.

When the same subjects returned for the meal intervention study, the same procedures were followed. At arrival, blood was drawn from the fasted subjects. They were then

randomly presented with a high or a low glycaemic index pre-exercise meal. The maximal exercise activity was started at 60 minutes post-meal and after blood sampling was performed.

4.4.3.2 Calculation of the maximal physical work capacity (PWC_{max})-index

Cardiorespiratory endurance is defined as the ability to perform large muscle, dynamic, moderate-to-high intensity exercise for prolonged periods. Performance of such exercise depends on the functional capacity/state of the respiratory, cardiovascular and skeletal muscle systems (ACSM, 1995). The traditionally accepted criterion for measuring cardiorespiratory endurance is directly measured maximal oxygen uptake (Vo_{2max}). This measurement is not always feasible; therefore, procedures for estimation of Vo_{2max} have been developed (ACSM, 1995), which were used in this study.

The calculation may be done by either plotting the heart rate (bpm) against the estimated $\dot{V}O_2$ (ml/min), or substituting the VO_2 by the exercise intensity ($\dot{w}$att) as illustrated in Figure 4.2. The obtained heart rates were then extrapolated to the age predicted maximal heart rate. A line vertical to the intensity scale estimates the maximal exercise intensity from which an estimated $\dot{V}O_{2max}$ or PWC_{max}-index can be calculated (ACSM, 1995).

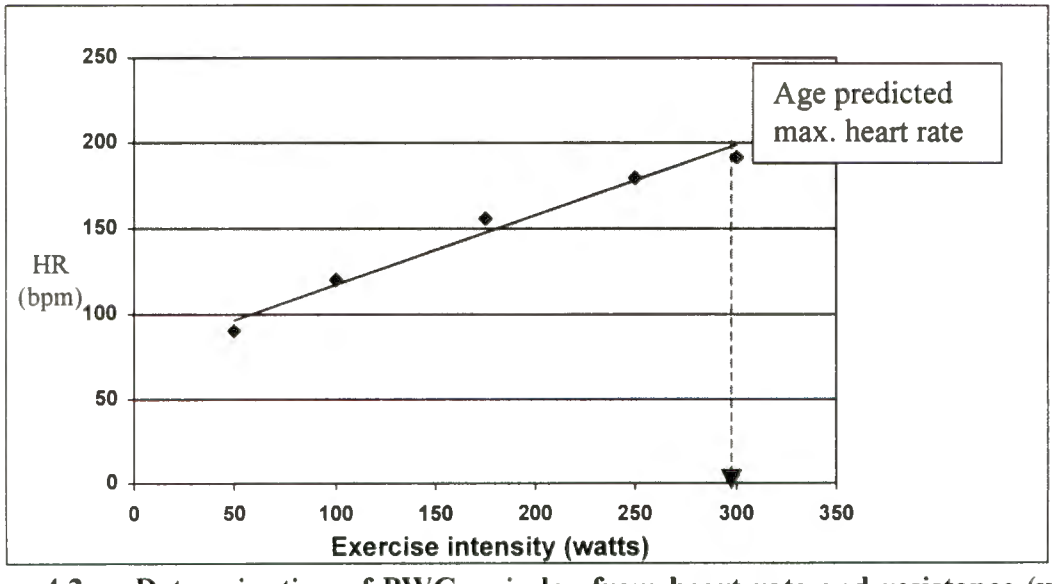

Figure 4.2 Determination of PWC_{max}-index from heart rate and resistance (watt) (ACSM, 1995)
HR = heart rate; bpm = beats/min; PWC_{max} = physical work capacity at maximal resistance

4.4.4 Meal interventions

4.4.4.1 Baseline

No meal intervention was done during baseline testing. The subjects followed their normal diet and reported fasting on the morning of testing. The subjects were only allowed to take water.

4.4.4.2 Pre-glycaemic intervention evening meal

A standard meal was prepared for the subjects on the evening preceding the glycaemic meal intervention. This meal consisted of a balanced but low-fibre meal to eliminate interference in the glucose response to the pre-exercise meal on the following day through the "second meal effect" (Wolever, 1990). All subjects were given the same meal. To compile this pre-fasting meal for all the subjects, a 24-hour recall method (Addendum D) was used to determine daily intakes. From this data an average energy intake and energy distribution (percentage fat, protein and carbohydrates) of the diet were determined. This average energy intake was divided by three to calculate the average energy intake per meal, using three meals per day as the norm.

Average daily energy (kJ) intake: 12 366
Estimated energy (kJ) per meal: 12 366 ÷ 3 = 4 122kJ

The energy distribution of the pre-fasting meal was a combination of the subjects' existing dietary pattern (average distribution according to the 24 hour recall) and the distribution recommended for a prudent diet (Ralph, 1993) The composition of this meal is given in Table 4.2.

Table 4.2: The composition and energy distribution of the pre-fasting meal served to the active and sedentary males*

Amount	Food source	Carbohydrate(g)	Protein(g)	Fat(g)	Energy(kJ)
250ml	Low fat milk	12.3	8.5	0.5	373
80g	Peeled apple	10.3	0.2	0.2	186
60g	Cheese (Gouda)	1.3	14.9	16.4	899
30g	Jam	30.0	-	-	510
20g	Margarine (Canola)	-	-	16.3	619
5 × 30g	White bread	74.0	12.8	2.7	1 578
TOTAL (g)		**127.9**	**36.4**	**36.1**	
kJ		**2 174**	**619**	**1 372**	**4 165**
% of kJ		**52.20%**	**14.86%**	**32.94%**	

*** calculated according to the South African Food Composition Tables (Mahan & Escott-Stump, 1996)**

4.4.4.3 Glycaemic index (GI) pre-exercise meal interventions

As discussed in the previous chapter, the hypothesis being tested is that the metabolic composition of the plasma may influence the fibrin network structures that will be formed. Therefore, high and low GI pre-exercise meal interventions were selected to create different metabolic "environments". The high GI meal (Table 4.3), results in a rapid digestion and absorption of carbohydrates. The reasons for the specific composition of the meal, are the following (Joint FAO/WHO Expert Consultation, 1997):

- Hot porridge was given due to the smaller amount of resistant starch, which will not lower the GI, as in the case of cooled cooked starch.
- Starch is maximally gelatinised in soft porridge
- The small particle size of refined maize meal favours digestion
- Glucose has a higher glycaemic index than sucrose
- The glycaemic index of this meal was 64%, as obtained during a pilot study using glucose as standard (Pieters, 1999)

Table 4.3: The composition of the high glycaemic index pre-exercise meal together with the preparation*

High Glycaemic Index (GI) meal (Warm maize porridge)			
FOOD INGREDIENTS		CHO CONTENT	METHODS
50g	Refined maize meal	40g*	Microwave water for two minutes
285ml	Water		Add maize meal and salt while stirring
5ml	Salt		Microwave porridge for 2 min. Stir well
10g	Glucose	10g	Microwave for 1min. Stir well.
			Add glucose and serve immediately.
TOTAL = 50g Carbohydrate			

***Englyst & Pieters, 1998**

The low GI meal as indicated in Table 4.4, was planned to not digest as rapidly as the high GI meal due to changes that occur in the structure of the starch when it cools down. This causes digestion to slow and starch that escapes digestion is fermented in the colon, producing short chain fatty acids and not glucose. Glucose is then released at a slower and more constant rate creating a different internal environment than during fasting or the high GI meal. Reasons for the meal composition were the following (Joint FAO/WHO Expert Consultation, 1997):

- Cooled cooked starch contains high amounts of resistant starch that lowers the glycaemic response.
- The bigger particle size of samp slows digestion and leads to lower glycaemic response, because these particles are less accessible to digestive enzymes.
- Sucrose has a glycaemic response of half that of glucose. 10g of sucrose contains ± 5g of glucose and 5g of fructose. Fructose has very little effect on the glycaemic response. The remaining 5g of glucose can influence the glycaemic response.
- The glycaemic index of this meal was 40% during the same pilot study, as mentioned previously, on the same subjects (Pieters, 1999).

Table 4.4: The composition of the low glycaemic index pre-exercise meal together with the preparation*

Low Glycaemic Index (GI) meal (Cold samp porridge)			
FOOD	INGREDIENTS	CHO CONTENT	METHOD
46,6g	Raw samp	40g*	Soak samp in 300ml of boiled water overnight
300ml	Water		Cook samp 2h45min. at low to medium heat. Stir occasionally.
5ml	Salt		Add salt when samp is almost cooked.
10g	Sugar	10g	Leave to cool Serve cold with 10g sugar
TOTAL = 50g Carbohydrates			

*** Englyst & Pieters, 1998**

4.4.5 Blood sampling

4.4.5.1 Samples for blood, serum and plasma analyses

The nursing sister drew blood samples from the *vena cepahalica*, using a sterile butterfly infusion set (Johnson & Johnson, 21G, 19mm) and syringes. For the preparation of serum, 20 ml of blood was allowed to clot in glass tubes (Vacutainer®, SST®). These tubes were then centrifuged at 4 000 rpm (Universal 16R™, HETTICH) for 15 min at 10°C. The serum was divided into four aliquots in 1.5 ml Eppendorf® vials for each subject and frozen until needed for analyses at -84°C.

Five millilitres of blood was transferred to a sodium fluoride potassium oxalate tube for the determination of the glucose. This was centrifuged at 3 500 rpm at 10°C for 10 min. The serum was divided into Eppendorf® vials and kept at -84°C until analysed.

For the preparation of the plasma, venous blood was mixed with a 3.8% sodium citrate (pH 4.5 – 4.8) buffer in the ratio of 9:1 in sterile plastic syringes. The citrated blood samples were centrifuged at 2 500 rpm at 10°C for 15 minutes. The citrate plasma was divided into seven aliquots and stored at -84°C in 1.5 ml Eppendorf® vials.

To determine the haematocrit and haemoglobin, EDTA blood was needed. Five milliliters of blood was transferred to K_3EDTA tubes (VAC-U-TEST®). The blood cells and the EDTA plasma were separated by centrifugation at 3 500 rpm for 10 minutes at 10°C. The aliquots of plasma were stored at -84°C and the cells stored separately at -20°C.

4.4.5.2 Blood samples for fibrin network structure studies

These blood samples were collected between 6:00 and 10:00 am at the same time as the other samples by means of the sterile butterfly infusion set (Johnson & Johnson, 21G, 19mm). Thirty millilitres of blood was drawn without stasis from the *vena cepahalica*. The venous blood was mixed with 3.8% tri-sodium citrate (pH 7.5) in the ratio 9:1 in plastic syringes. The blood was transferred to sterile plastic Falcon tubes which contained 35 µl of 10 000 KIU/ml aprotinin (Trasylol®, Bayer-Miles, Germany) for every 10 ml of citrated blood. The citrate prevents early activation of factor V and VII, while aprotinin inhibits fibrinolysis. The samples were immediately thoroughly mixed, after which platelet-poor plasma (PPP) was obtained by centrifugation at 3 660 rpm in a *Mistral 3000E* centrifuge (Sanyo, England) for 20 minutes. The platelet-poor plasma was transferred to clean sterile falcon tubes and once more centrifuged at 3 660 rpm in the same centrifuge for 15 minutes to obtain essentially platelet-free plasma (E-PFP). The platelet-free plasma was then kept at -84°C for less than 6 months (McNerlan *et al.*, 1997) until the fibrin network structure characteristic analyses were performed.

4.4.6 Biochemical analyses of blood, plasma and serum

The biochemical variables that were analysed in the blood, plasma and serum during this study are shown in Table 4.5. The normal ranges, as indicated by the specific method, the reference, and the apparatus used, are also given, together with the location of where the analyses were performed.

Table 4.5: The biochemical methods for analyses of blood, serum and plasma samples of the subjects together with the normal range, method and references, location and apparatus

Variable	Normal range	Method & Reference	Location	Apparatus
BLOOD				
Haematocrit	39 – 49% (males)	Capillary tube (Marienfeld, Germany) Haematocrit centrifuge	Research Laboratory, Department Nutrition and Family Ecology, PU for CHE, Potchefstroom	(Hettich Zentrifugen Haematocrit 24, D-78532, Tutlingen)
SERUM				
Sodium	137 – 144mmol/L	DAX Profile (Discrete analyzer)	Department of Chemical Pathology University of Pretoria	LX 20 from Beckman OF Technicon DAX 48
Potassium	3.6 – 4.7 mmo/L			
Chloride	98 – 108 mmol/L			
Total CO_2	23 – 29 mmol/L			
Anion gap	7 – 14 mmol/L			
Urea	3.1 – 7.8 mmol/L			
Creatinine	81 – 114 mmol/L			
Uric acid	0.31–0.47mmol/L			
Total calcium	2.2 2.55 mmol/L			
Correlated calcium	2.2 2.55 mmol/L			
Magnesium	0.7 – 0.95mmol/L			
Phosphate	0.87–1.45mmol/L			
Total protein	66 – 79 g/L			
Albumin	39 – 50 g/L			
Globulin	18 – 36 g/L			
Total billirubin	4 – 30 µmol/L			
Unconjugated billirubin	2 – 14 µmol/L			
Conjugated billirubin	0 – 8 µmol/L			
Alanine phosphatase	38 – 102 IU/L			
γ-gluthamyl transferase	8 – 32 IU/L			
Alanine transferase	6 – 32 IU/L			
Aspartate transferase	9 – 34 IU/L			
Lactate dehydrogenase	90 – 180 IU/L			
Total cholesterol	3.0 – 5.2 mmol/L			

LDL-Cholesterol HDL-Cholesterol Triacylglycerol Glucose Osmolarity	2.0 – 3.4 mmol/L 0.9 – 1.6 mmol/L 0.8 – 1.5 mmol/L 3.9 – 5.8 mmol/L 275–295 mmol/L			
Albumin	34 – 50 g/L	Boehringer Mannheim; Serum albumin binds quantitatively to 5,5-dibromo-ϕ-cresol-sulphenophethalein (BCP) Cat no. 1489143 *Control Precipath U (Cat. No. 171760)* (CV = 3.23%) *Control Precinorm U (Cat. No. 171735)* (CV = 3.01%)	Fibrinogen Unit Technicon Free State, Bloemfontein	Kinetic reader *EL312UB* (Biotec Instruments)
Total protein	55 – 83 g/L	Boehringer Mannheim; Cat. No. 1553836 *Control Precipath (U Cat. No. 171760)* (CV = 3.1%) *Control Precinorm U (Cat. No. 171735)* (CV = 2.5%)	Fibrinogen Unit, Technicon Free State, Bloemfontein	Kinetic reader *EL312UB* (Biotec Instruments)
Triacylglycerol	≤ 2.3 mmol/L	Boehringer Mannheim; Enzymatic hydrolysis of triacylglycerol with subsequent determination of liberated glycerol by colorimetry Cat. No. 1488872 *Control Precipath U (Cat. No. 171760)* (CV = 3.16%) *Control Precinorm U (Cat. No. 171735)* (CV = 1.9%)	Fibrinogen Unit, Technicon Free State, Bloemfontein	Kinetic reader *EL312UB* (Biotec Instruments)
Glucose	4.2 – 6.4 mmol/L	After enzymatic oxidation in presence of glucose oxidase. Boehringer Manheim; Bromocresol purple method(BCP) Cat.No.GL2623 *Control Precipath U (Cat. No. 171760)* (CV = 2.81%) *Control Precinorm U (Cat. No. 171735)* (CV = 2.75%)	Fibrinogen Unit, Technicon Free State, Bloemfontein	Kinetic reader *EL312UB* (Biotec Instruments)

PLASMA				
D-dimer	4 – 78 µg/L	ELISA method enzygnost®, D-dimer micro wavelength, 492 Behring, Cat. No.OQBC11 CV = 3.57%	Fibrinogen Unit, Technicon Free State, Bloemfontein	Spectrophotometer UV – 1201 Shimadzu (wavelength 405nm)
Fibrin monomer	<3.4 – 14.5 mg/L	Berichrom® FM Kinetic method (enzyme reaction). Soluble fibrin in the sample stimulates activation of plasminogen to plasmin by tissue	Fibrinogen Unit, Technicon Free State, Bloemfontein	Spectrophotometer UV – 1201 Shimadzu (wavelength 405nm)
C-reactive protein	< 6 mg/L	The sample is reacted with specific antiserum. The formed precipitate is measured turbidimetrically at 340nm. Boehringer Mannheim; Bromocresol Purple method (BCP) Cat. No. CP788 *Control serum* CV = 0.87%	Fibrinogen Unit, Technicon Free State, Bloemfontein	Kinetic reader *EL312UB* (Biotec Instruments)
Total antioxidant status	1.3–1.77 mmol/L	Boehringer Mannheim; Bromocresol Purple method (BCP) Cat. No. NX2332 *Control serum (Cat. No. NX2331)* (CV = 1.79%)	Fibrinogen Unit, Technicon Free State, Bloemfontein	Kinetic reader *EL312UB* (Biotec Instruments
Insulin	72 – 179 ρmol/L (10 – 24 µU/mL	Radioimmunoassay for the quantitative determination of insulin concentration, Radiolabelled insulin competes with unlabelled insulin for binding sites on anti-insulin immobilised to the inside wall of the tube. (Cat. No. IC13021) *Control serum* CV = 25.85%	Departement of Physiology, PU for CHE, Potchefstroom	Packard Cobra Auto Gamma Counter
Factor VII	50 – 150%	Plasma FVIIc with one-stage clotting assay, calcium thromboplastin (PT-Fib kit) Instrumentation Laboratories, Milan (Cat. No. 84682-10) CV = 4.12%	Research Laboratory, Department of Nutrition and Family Ecology, PU for CHE, Potchefstroom	ACL200 (Automated Coagulation Laboratory)
Fibrinogen	2.0 – 4.0 g/L	Functional measured with the Clauss method Reagents from Instrumentation Laboratories, Milan. (Cat. No. 1-084691-10) CV = 8.24%	Research Laboratory, Department of Nutrition and Family Ecology, PU for CHE, Potchefstroom	ACL200 (Automated Coagulation Laboratory)

4.4.7 Determination of fibrin network structure characteristics

4.4.7.1 Preparation of fibrin networks

In order to form fibrin networks from the collected plasma, various preparations had to be done. The fibrin networks were formed in disposable plastic cuvettes that were adapted by the Technical Department of the PU for CHE. These cuvettes with a 1 cm-path length were adapted by drilling a hole at the base of the cuvette to enable percolation through the fibrin networks during permeation experiments.

To form the fibrin networks with the plasma in the disposable cuvette, the outlet were sealed off with a rubber plug. The cuvettes were then coated with a fibrinogen solution (2g/1 000 ml). The fibrinogen coat enabled the network to adhere to the cuvette, preventing the network from collapsing, without influencing the measurements. The cuvette was left overnight to dry sufficiently before the fibrin networks were formed.

Two millilitre of the platelet-free plasma was pipetted into the coated disposable cuvettes. To initiate fibrin network formation, 40 µl of a 1 M calcium chloride solution (20 mM) together with 23 µl of a 20 IU/ml thrombin solution (0.23 IU/ml) were added to the platelet-free plasma. The solution was carefully but thoroughly mixed and sealed off by a specially designed perspex flow-through stopper with a silk net, as described by Blombäck *et al.* (1994a) and Fatah *et al.* (1996a) and as illustrated in Figure 4.3. Care was taken to ensure that no bubbles were formed in the solution and that the solution made contact with the silk net. Excess plasma above the silk net was withdrawn by means of a glass pipette. The fibrin networks were left for two hours at room temperature to ensure gelation. A 0.02 M Tris-imidazole buffer (0.02 M Trisma base, 0.02 M Imidazole and 0.1 M NaCl, pH 7.4, adjusted with 0.2 M HCl solution) were added *via* the stopper inlet to prevent the fibrin networks from dehydrating.

The networks were left for 18 to 24 hours for complete polymerisation. During this period, the total fibrinogen content in the plasma was incorporated into the network structure. The fibrin networks were made in triplicate for each sample of each subject.

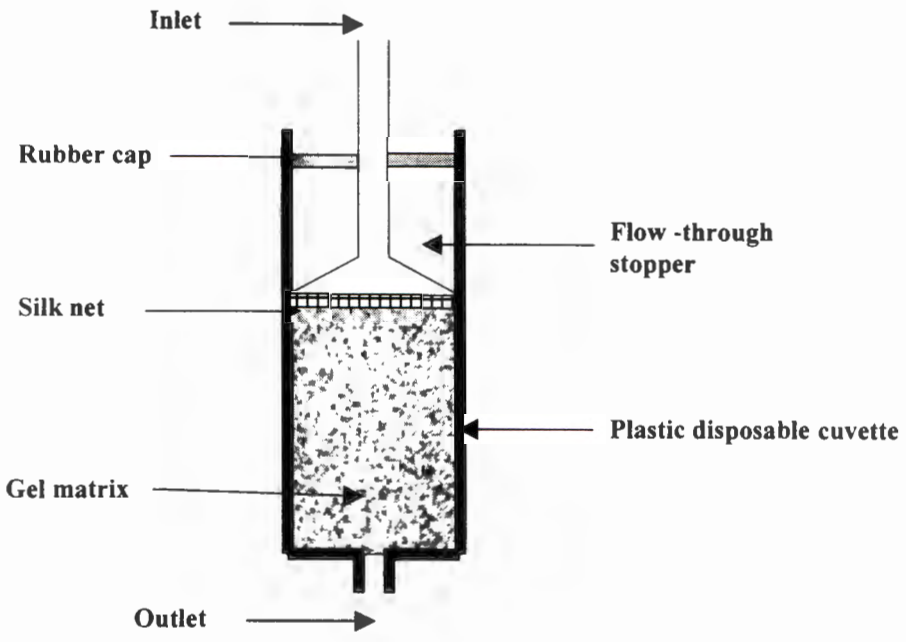

Figure 4.3 The disposable plastic cuvette for percolation and turbidity measurements of fibrin gels (Blombäck *et al.*, 1994a)

4.4.7.2 Determination of mass-length ratio (MLR, μ_T)

After the fibrin, networks had been left for 18 to 24 hours for total polymerisation, the mass-length ratio of the networks was determined. The turbidity (optical density × 2.303) of the fibrin network was recorded by measuring the optical density of the network with unclotted plasma in the reference cell, at intervals of 2 nm, between wavelengths of 600 nm to 800 nm (*Shimadzu UV-2100 Visible Recording Spectrometer*).

The mass-length ratio from the optical density was calculated as follows: A plot was drawn with $c/T\lambda^3$ as a function of $1/\lambda^2$, where T is turbidity (2.303 × optical density), λ is the wavelength and c is the concentration of fibrinogen in mg/ml. The intercept of A on the y-axis, of this linear plot, was used to calculate the average mass-length ratio, μ_T, according to the following equation as described by Carr and Hermans (1978) and Nair *et al.*, (1991a):

$$\mu_T = \frac{10}{1.48.A} \times 10^{12} \text{ daltons/cm}$$

4.4.7.3 Determination of permeability coefficient (K_s)

The fibrin network samples prepared in section 4.4.6.1, were used in the determination of the mass-length ratio as well as the network permeation experiments (K_s). To determine the permeability of the fibrin network structure, the 0.02 M Tris-imidazole buffer was percolated through the fibrin network structure that had been placed in the apparatus as illustrated in Figure 4.4 (Fatah *et al.*, 1996a). The Technical Department of the PU for CHE developed the apparatus.

After the network had been placed in the apparatus, the buffer was percolated at 5 different hydrostatic pressures above the network. To allow for percolation through the fibrin network, the rubber plug was carefully removed from the bottom of the disposable cuvette. A piece of plastic tubing was placed in the position of the rubber plug to serve as an outlet for the elute. Percolating the buffer through at 7 cm above the network, washed it until the networks were a white colour. This was to ensure that any protein that might be caught in the structure was washed out. The elute for each pressure was collected to determine the volume of buffer percolated. The corresponding time in seconds it took for each volume of elute was also recorded. Finally, the temperature of the buffer was measured throughout the permeation, as the temperature influences the viscosity of the buffer. With all the above-mentioned measurements recorded, the permeability coefficient (K_s) was calculated according to the formula described by Blombäck and Okada (1982).

$$K_s = \frac{Q \times L \times \mu}{t \times A \times \Delta P}$$

Where Q (in cm^3) is the volume of the liquid having the viscosity μ_T (in poise.dyne × $sec./cm^2$) flowing through a chamber length L (in cm). The area of the chamber is A (in

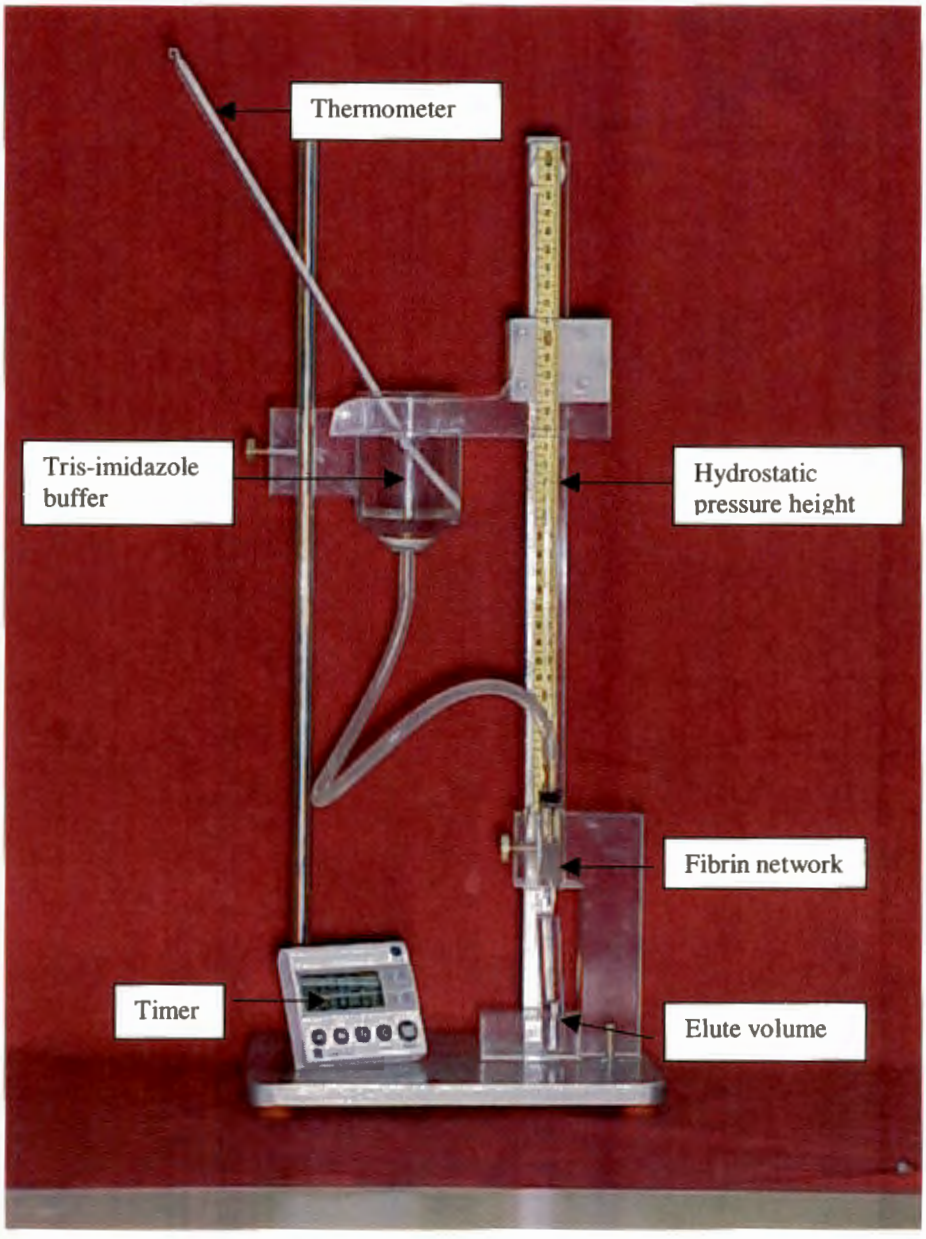

Figure 4.4 **A photograph of the apparatus developed for the fibrin network permeation experiment (K_s). The disposable plastic cuvette was placed in the apparatus with the 0.02 M Tris-imidazole buffer percolating through the network at different heights**

cm^2), with t the time (in sec.) for the liquid passing through the chamber under a pressure gradient P (in dyne/cm^2). The viscosity of the buffer was determined by means of a Brookfield digital viscometer (Model DV-1+, Version 3.0, Stoughton, USA) at temperatures between 10°C and 30°C. The density of the buffer was determined by means of a picnometer (Merck, Germany) for the determination of the volume of the accurately weighed perforate.

4.4.7.4 Compaction of fibrin network structures

The method of compaction of the fibrin network structures is based on the number and strength of the primary crosslinks and branch points in the network (Nair *et al.*, 1991a). The compaction was measured in quadruplicate using the method of Dhall *et al.* (1976) and Nair and Shats (1997). This method describes the tensile strength of the fibrin fibers.

The fibrin networks were formed in 1.5 ml Eppendorf® vials. These vials were sprayed with a lecithin-based aerosol (Spray and Cook®, Colman Foods, South Africa) to ensure that the fibrin network formed does not adhere to the surface. One millilitre of the platelet-free plasma was pipetted into the vials. The fibrin networks were formed by addition of 20 mM $CaCl_2$/ml and 0.23 IU/ml of thrombin. The vials were left for at least 12 hours for complete polymerisation.

The vials with the fibrin networks were centrifuged the following day for 45 sec. at 8 000g in an Eppendorf centrifuge (model 5415C, West Germany). The supernatant expelled was measured by drawing it off with an insulin syringe. The supernatant was measured and expressed as a percentage of the initial volume of the network to give an index of the collapsibility of the network.

4.4.8 Coefficient of variation for fibrin network structure studies

The coefficient of variation (CV) for the measurements of fibrin network structure characteristics was determined on a pooled plasma obtained from a random group of 40

volunteers recruited statistically from a list of permanent male personnel of the PU for CHE. Blood was drawn from the subjects as described in section 4.4.5. The plasma was obtained as described in section 4.4.7.

The fibrin network structure experiments were done on the pooled plasma on five occasions with a minimum of five samples per day. This was done before the experiments on the plasma of the subjects that participated in the project.

The CV was calculated as follows:

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean}} \times 100$$

The results of the CV for the different methods of fibrin network structure characteristics are summarised in Table 4.6.

Table 4.6: The CV of the various methods for the characterisation of the fibrin network structure

Methods for Fibrin network structure characterisation	CV (%)
Mass-length ratio (MLR) from turbidity (μ_T)	10.20% (n = 45)
Permeability (K_s)	10.74% (n = 45)
Compaction (%)	7.64% (n = 45)
Fibrinogen (g/L)	8.24% (n = 28)

4.5 Statistical analyses

All statistical analyses were performed with the SPSS system for Windows® (1999) programme. The means and standard deviations of the various parameters were obtained by means of descriptive statistics. To determine whether there were significant differences between the active and sedentary groups, a non-parametric independent t-test was performed. For determining significant differences within the groups during the various interventions, non-parametric dependent t-tests were performed. The p-value for significance were set at $p \leq 0.05$. Spearman correlation coefficients between the changes in metabolic variables at the different times and the changes in the fibrin network structure over the same time were calculated with the same programme. Fifteen percent of all the variables showed a significant correlation ($r \geq 0.50$; $p \leq 0.05$).

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Introduction

The literature background and methods used have been discussed extensively in the previous chapters. In this chapter the results obtained will be presented in the form of two prepared manuscripts. The first manuscript will report changes in fibrin network structure characteristics of fasting active and sedentary males after maximal exercise. Some biochemical variables are reported to support the discussion on the fibrin network structure characteristics. Additional variables that have been determined and not included in the manuscript, are presented in Addendum E.

The second manuscript will discuss the changes in fibrin network structure of active and sedentary males with regard to the influence of a high and low GI pre-exercise meal. A few selected biochemical variables will also be discussed together with the fibrin network structure characteristics. Additional variables measured during this part of the study are reported in Addendum E. The results of the Spearman's correlation coefficients between changes in serum variables and changes in fibrin network structure characteristics are given in Addendum F.

Fibrin Network Structure: The response to maximal exercise in sedentary and active male subjects

Sarah J. Moss[1], Maides M. Malan[2], Lukas I. Dreyer[1], & Hester H. Vorster[3]

[1]Institute for Biokinetics at the School of Biokinetics, Recreation and Sport Science

[2]The School of Pharmacy

[3]The School of Physiology, Nutrition and Family Ecology

Potchefstroom University for Christian Higher Education

Potchefstroom, South Africa.

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Abstract

The mechanism through which regular physical activity reduces the risk of cardiovascular disease (CVD) is not clear. The independent, probably causal, association of increased plasma fibrinogen with CVD is established. It is hypothesised that part of this association may be mediated by the quality of fibrin network structures formed when fibrin(ogen) monomers polymerise. The effect of physical activity on network structure is not known.

The objective of this study was to monitor fibrin network characteristics during maximal physical activity on a bicycle ergometer and after a recovery period of 30 minutes in highly active and sedentary young men. In response to the maximal activity fibrin network structures formed from plasma of the active group were less permeable and had higher tensile strengths than at rest, and changed to more permeable networks with lower tensile strengths after 30 minutes of recovery. In the sedentary group fibrin network structures with higher tensile strengths and less permeability were formed after 30 minutes of recovery from a maximal activity when compared to the networks formed during maximal activity. It is concluded that the effects of physical fitness and acute bouts of intensive exercise on fibrin networks formed from plasma of healthy young males may differ, but that more research is needed to understand underlying mechanisms and relate these differences to health outcomes.

Key words: Fibrin network structure, maximal exercise, health, males

Introduction

Plasma fibrinogen is accepted as a major cardiovascular risk factor. A number of prospective epidemiological studies indicated that plasma fibrinogen levels are strongly predictive of fatal and non-fatal coronary heart disease (CHD), myocardial infarction, and stroke events [1]. Of the various haemostatic determinants, the association of fibrinogen with an increased risk of CVD is the most consistent and powerful [2]. The positive relation between plasma fibrinogen and CHD seems to be independent of other risk factors [3]. All the pathophysiological mechanisms through which a high level of plasma fibrinogen promotes atherogenesis are, however, still uncertain [4]. Recent developments in the study of haemostasis showed that not only the fibrinogen concentration, but also the quality of fibrin networks, might be a risk factor for the development of CVD [5, 6].

Plasma fibrinogen levels have a direct influence on the fibrin network structure [7] but a significant characteristic is that the quality of the structure may be influenced by a complex interplay of fibrinogen and other risk factors. This interplay may lead to changes in the polymerisation conditions. According to Blombäck *et al.* [8] the polymerisation conditions are determined by kinetic and modulating factors. The kinetic factors are thrombin and fibrinogen concentrations. The modulating factors such as protein like albumin, fibronectin, specific ions like calcium and the total ionic strength [9] affect the structures as they are being formed with otherwise constant kinetic factors. Increased levels of the kinetic factors will result in tighter, less porous networks with thinner fibres and higher density nodes. Flow through structures is impaired [10]. Conversely, low concentrations of kinetic factors result in porous networks with thick fibres and fewer nodes. Advances in the methodology have made it possible to determine the different types of structures that are formed by turbidity [11] and permeability [12] techniques. Patients suffering from cardiovascular disease are prone to the formation of tight and rigid fibrin network structures reflected in smaller mass-length ratios (MLR) of fibres calculated from turbidity and lower permeability coefficients (K_s) of the fibrin clots [7].

The health benefits of exercise have long been recognised. As early as 1899 physicians have recommended physical activity to protect against postoperative deep vein thrombosis [13]. Although the effects of exercise on coronary risk factors such as lipid profiles have

been extensively studied, the changes that occur in the haemostatic system during long-term exercise leading to physical fitness as well as acute exercise, have been more difficult to establish. Several studies have found an inverse relationship between physical activity and plasma fibrinogen [14 - 17]. Compared to this long-term beneficial effect, the effects of acute or once-off maximal activity on fibrinogen levels seem contradictory. De Scalzi *et al.* [18] showed an elevation in plasma fibrinogen, which suggests an increase in risk for stroke and or a myocardial infarction during an once-off activity of high intensity. The changes in the fibrin network structure formed from plasma obtained during and after exercise in man are virtually unknown.

The present study was designed to determine the influence of maximal exercise on the fibrin network structure of sedentary and fit, highly active males. The fibrin network structure was investigated by porosity characteristics (permeability coefficient, K_s) and fibre MLR of the native fibrin network structure formed *in vitro* in plasma obtained from young active and sedentary males.

Materials and Methods

Subjects

A total of 29 young, healthy males between the ages of 19 and 30 years and with a body mass index (BMI) of 18 – 25 kg/m^2 were recruited to voluntarily participate in the study which was approved by the Ethics Committee of the University of Potchefstroom for Christian Higher Education. Fifteen of the subjects were highly active (elite athletes) and 14 were sedentary (participating in no organised physical activity). The active males ran between 30 and 60 km/week and all of them had a best 10 km time trail of less than 40 minutes during the preceding 3 months. None of the subjects had any chronic disease, smoked or drank alcohol. No medication or supplements were taken 10 days before testing, as recommended by Van den Burg *et al.* [19]. All respondents fasted for 12 hours before testing and abstained from physical activity during the previous 24 hours.

Blood sampling

Venous blood samples were taken between 7:30 and 10:00 am as recommended by Szymanski *et al.* [20] to accommodate the changes in plasma fibrinogen levels during the day. Blood was collected without stasis using atraumatic venepuncture by means of a sterile butterfly infusion set into plastic syringes with 1 part 3.8% tri-sodium citrate and 9 parts of blood with 35 µl of 10 000 KIU/ml Trasylol® /aprotinin (Bayer, Germany) [23]. The subjects fasted overnight (12h) to avoid interference of turbidity measurement by abnormally elevated plasma lipids. Plasma was immediately prepared by centrifugation at 3660 rpm for 15 min at room temperature to obtain platelet-poor plasma, which was again centrifuged for 10 min to obtain essential platelet-free plasma. The plasma was dispensed into plastic tubes and stored at -80°C for less than 3 months until the fibrin network characterisation experiments were performed.

Maximal exercise procedure

Twenty millilitres of blood was drawn before the subjects were subjected to a maximal exercise session. The resting blood pressure and heart rate were monitored. The subjects were subjected to maximal exertion on a Monark Bicycle ergometer. The test was started by using a 50-watt resistance at 70 rpm [18]. After every three minutes the heart rate (*Cardio Sport*®) and blood pressure (sphygmomanometer) was monitored in order to increase the resistance by 50 watt every four minutes when a steady state in the heart rate was obtained. The resistance was increased until the maximum age-adapted heart rate [21] was obtained, or until the subjects experienced maximum exertion as expressed by the rate of perceived exertion [22]. The exercise was conducted inside an air-conditioned laboratory with the temperature maintained at 23°C. The second blood sample was drawn within 5 min of reaching the maximum heart rate, or alternatively exhaustion. The final blood sample was drawn after a 30-min recovery period in a stationary position.

The *Physical Work Capacity* (PWC_{max}) for each individual subject was determined by plotting the heart rate against the resistance (watt) [21]. From this graph the maximum resistance was determined and expressed in relationship to the mass (kg) of the subject.

This determination was based on the assumption that a linear relationship exists between the heart rate and the resistance at which activity is performed. The more physically conditioned the subject, the higher the resistance will be before exhaustion is reached.

Network permeation experiment (Permeability coefficient, K_s)

The fibrin network structures were formed in a disposable fibrinogen pre-coated cuvette after a final concentration of 25 mM $CaCl_2$ and 20 IU thrombin was added to the plasma. The cuvette was closed with a plastic stopper covered with silk. The fibrinogen coating together with the silk was to ensure that networks adhere to the internal surface of the cuvette and do not collapse. The fibrin networks were left to gel for two hours after which Tris-buffer was added to prevent the networks from dehydrating. Percolation of the network structures was performed only after 24 hours to ensure complete polymerisation. The porosity of the fibrin network structures were determined by percolating a buffer (0.02 M Tris-imidazole, 0.01 M NaCl, pH 7.4) at five different hydrostatic pressures through the gel for a recorded time (seconds). The elutes were collected to determine the volume percolated. The permeability coefficient (K_s) was calculated as previously described [12] according to equation 1.

$$K_s = \frac{Q \times L \times \mu}{t \times A \times \Delta P} \quad \text{.....(1)}$$

where Q (in cm^3) is the volume of the liquid having the viscosity μ (in poise.dyne x s/cm^2) flowing through a chamber of the length L (in cm). A is the area (in cm^2) of the chamber and t is the time (in s) for passing of the liquid through the chamber under a pressure gradient P (in $dyne/cm^2$). The CV for the method was 10.74%.

Mass-length ratio determinations (MLR, μ_T)

MLR was determined by the turbidity technique where turbidity (μ_T) was calculated as optical density multiplied by 2.303. The fibrin network structures were formed in

disposable cuvettes as for the permeation technique. The turbidity of the networks was recorded with intervals of 2nm over a wavelength range of between 600nm and 800nm (*Shimadzu UV-2100 Visible Recording Spectrometer*) with unclotted plasma in the reference cell. $c/T\lambda^3$ was plotted as a function of the $1/\lambda^2$, where T is the turbidity (2.303 x optical density), λ is the wavelength and c is the concentration of fibrinogen in mg/ml. The intercept A of this plot was used to calculate the average mass-length ratio, μ_T, according to the equation 2 [11, 24]. The CV for the method was 10.20%.

$$\mu_T = \frac{10}{1.48A} \times 10^{12} \text{ daltons/cm} \quad \text{.....(2)}$$

Measurement of plasma fibrinogen and Factor VII concentrations

Functional plasma fibrinogen concentration was determined by an adapted Clauss method [25] using an *ACL 200*™ automated coagulation analyser and reagents from Instrumentation Laboratories (IL) (Milan, Italy). Fibrinogen standards and controls were purchased from IL. The CV for the method was 8.24%.

Plasma Factor VII was determined with a one-stage clotting assay on an ACL 200 with a PT-Fib kit from IL (Milan, Italy). The CV for the method was 4.12%.

Haematocrit

EDTA blood was used to determine haematocrit in a haematocrit centrifuge (Hettich Zentrifugen Haematocrit, Tutlingen).

Blood biochemistry

Serum albumin, total protein, triacylglycerol and glucose concentrations were determined with the relevant Boehringer Mannhein kits on a Kinetic reader (Biotec Instruments). A

CV of less than 3% was found for all the methods. The determination of the plasma D-dimer was performed by means of an ELISA method enzygnost® and had a CV of 3.57%. Fibrin monomer levels were determined with a Berichrome® FM Kinetic method. Both these determinations were performed with an UV Spectrophotometer (Shimadzu). Plasma levels of C-reactive protein and total antioxidant status (TAS) were determined with Boehringer Mannheim kits by means of a Kinetic reader (Biotec Instruments). The CV for the C-reactive protein was 0.87% while the CV for TAS was 1.79%. Insulin levels were determined by radioimmunoassay with a Packard Cobra Auto Gamma Counter and had a CV of 25.85%.

Statistical Analysis

All statistical analyses were performed with the SPSS system for Windows® (1999) programme. The means and standard deviations were determined by descriptive statistics. Non-parametric paired t-tests were performed to determine significant differences between baseline, maximal activity and 30 minutes of recovery for the sedentary and active groups respectively. All tests were considered as significant with a p-value of $p \leq 0.05$.

Results

Characteristics of the active and sedentary males at rest

The means and standard deviations of the personal characteristics of the two groups are given in Table 1. The active fit group and sedentary group were not matched for mass, BMI, percentage body fat or resting heart rate. Therefore, the sedentary group should not be seen as a control for the active, fit group, but rather as a separate experimental group. This means that within group, but not between group comparisons should be made.

The profile of the active group gives an indication of the effects of long-term physical training on body composition with regards to body mass (70.26 ± 8.33 kg), BMI (21.74 ± 1.79 kg/m^2) and percentage body fat (7.30 ± 1.07%). The low resting heart rate of the

active group (66.53 ± 12.93 bpm) is indicative of the active males being physically fit. In response to exercise the average maximal heart rate of the active group increased to 184.65 ± 7.87 bpm. The systolic blood pressure at rest was 125.59 ± 16.00 mm Hg with the diastolic pressure at 74.41 ± 9.50 mm Hg. The maximal exercise resulted in the increase of the systolic blood pressure (189.82 ± 19.76 mm Hg) and a decrease in the diastolic blood pressure (58.71 ± 9.16 mm Hg). The average resistance at which the active group exercised was 321.17 ± 52.84 watt. This resulted in an average *Physical Work Capacity* (PWC_{max}) of the active group of 4.36 ± 0.76 watt/kg.

Table 1: Means and standard deviations of the basic characteristics of the 15 active and 14 sedentary respondents including some metabolic variables determined in rest

	Active (n = 15)	Sedentary (n = 14)
Age (years)	21.80 ± 2.64	21.07 ± 1.71
Body mass (kg)	70.26 ± 8.33*	88.34 ± 17.69*
BMI (kg/m^2)	21.74 ± 1.79*	26.65 ± 5.54*
Percentage body fat(%)	7.30 ± 1.07*	17.18 ± 9.06*
Resting heart rate (bpm)	66.53 ± 12.93*	82.86 ± 13.16*
Resting systolic BP (mm Hg)	125.59 ± 16.00	132.08 ± 14.05
Resting diastolic BP (mm Hg)	74.41 ± 9.50	80.83 ± 7.93
S-TC (mmol/L)	3.70 ± 0.48*	4.81 ± 1.11*
S-LDL (mmol/L)	2.09 ± 0.60*	3.23 ± 1.06*
S-HDL (mmol/L)	1.27 ± 0.25*	1.07 ± 0.19*
S-TG (mmol/L)	0.76 ± 0.33*	1.17 ± 0.49*
S-Urea (mmol/L)	6.79 ± 1.21*	5.16 ± 1.06*
S-ALP (IU/L)	72.82 ± 26.99	84.40 ± 26.29
S-GGT (IU/L)	17.35 ± 4.22	22.89 ± 9.61
S-ALT (IU/L)	19.40 ± 13.44	25.84 ± 13.50
S-AST (IU/L)	33.16 ± 32.37	26.50 ± 9.03
S-LD (IU/L)	156.73 ± 44.24	147.03 ± 31.70
P-TAS (mmol/L)	1.24 ± 0.07	1.24 ± 0.08

BMI = body mass index; BP = blood pressure; TC = total cholesterol; LDL = low density lipoprotein; HDL = high density lipoprotein; TG = triglycerides; ALP = alanine phosphatase; GGT = γ-glutamyl transferase; ALT = alanine transferase; AST = aspartate transferase; LD = lactate dehydrogenase; TAS = total antioxidant status. * Significant difference between groups ($p \leq 0.05$)

The characteristics of the sedentary group were indicative of males not participating in regular physical activity. The body mass (88.34 ± 17.69 kg), BMI (26.65 ± 5.54 kg/m^2) and percentage body fat (17.18 ± 9.06%) were in the upper area of the normal range for healthy persons. The sedentary group had a resting heart rate of 82.86 ± 13.16 bpm that increased with maximal exercise to 182.64 ± 8.58 bpm. The resting systolic blood pressure of the sedentary group (132.08 ± 14.05 mm Hg) increased with maximal exercise to 193.00 ± 21.29 mm Hg. The diastolic blood pressure responded to maximal exercise by decreasing from rest (80.83 ± 7.93 mm Hg) to maximal exercise (67.57 ± 9.10 mm Hg). The average maximal resistance at which the sedentary group were able to exercise at, was 255.00 ± 34.81 watt. This gives an average *Physical Work Capacity* (PWC_{max}) of 3.30 ± 0.68 watt/kg for the sedentary group.

Table 2: Means and standard deviations of the physiological response of the active and sedentary respondents to maximal physical activity on a bicycle ergometer

	Active (n = 15)	Sedentary (n = 14)
Exhaustion heart rate (bpm)	184.65 ± 7.87	182.64 ± 8.58
Exhaustion systolic BP (mm Hg)	189.82 ± 19.76	193.00 ± 21.29
Exhaustion diastolic BP (mm Hg)	58.71 ± 9.16*	67.57 ± 9.10*
Maximal resistance (watt)	321.17 ± 52.84*	255.00 ± 34.81*
PWCmax (watt/kg)	4.35 ± 0.76*	3.30 ± 0.68*

PWC_{max} = physical working capacity at maximum beats per minute; BP = blood pressure
*** Significant difference between groups ($p \leq 0.05$)**

Fasting blood biochemistry variables of the active and sedentary males

The results of the blood biochemistry of the active males are reported in Table 1 and Table 3. The results indicate that the active males had total cholesterol, LDL-cholesterol, triglyceride, HDL-cholesterol, and glucose concentrations within the normal range, closer to the lower limits. The values for the liver enzymes were all within the normal range. This confirms that the active males were healthy. The fibrinogen values obtained with the active group were slightly higher than expected, but might have been due to oxidative stress caused by a high intensity-training regime in the preceding weeks. A correlation between elevated fibrinogen and oxidative stress has been reported [26]. This deduction is incorrect, as the total anti-oxidant status of the active group was determined as being within the normal ranges.

The results of the sedentary males as reported in Table 1 and Table 4 indicate that the sedentary males were healthy according to the liver enzymes that tested within the normal range. The results of the serum cholesterol, which included total cholesterol, LDL-cholesterol and HDL-cholesterol, were all within the normal range, but tend to be in the upper limits of the normal range. Similar findings were obtained with the triglyceride and glucose concentrations of the sedentary males.

Changes in the blood biochemical variables due to maximal activity and after 30 minutes of recovery from maximal activity

The results for active males obtained during rest, maximal activity and 30 minutes of recovery are given in Table 3. All the results of this study have been adapted for the shift that occurs in the plasma volume during physical activity. All the metabolic variables that were measured showed the same trend for the active males. From fasting to maximal activity a significant increase was found except for albumin. After 30 minutes of recovery, all the variables indicated a significant decrease from the values obtained at maximal activity. Total protein was the exception, as the decrease was not significant. All the values after 30 minutes of recovery was lower than the initial resting values, of which only the triglycerides were significantly different.

Table 3: Means and standard deviations of the changes in the plasma and serum biochemistry of some metabolic variables as measured at rest, maximal activity and 30 min of recovery of the active males

Parameters	Fasting	Max. activity	30 min recovery
Haematocrit (%)	44.13 ± 3.58*	46.33 ± 4.48* $^{\nabla}$	43.40 ± 3.96$^{\nabla}$
Albumin (g/L)	47.38 ± 2.15	53.05 ± 2.52$^{\nabla}$	49.14 ± 4.99$^{\nabla}$
Total protein (g/L)	69.52 ± 2.46*	66.51 ± 6.39*	68.94 ± 6.20
Glucose (mmol/L)	4.67 ± 0.36*	5.59 ± 0.73* $^{\nabla}$	4.46 ± 0.65$^{\nabla}$
Triglyceride (mmol/L)	0.76 ± 0.33* $^{\#}$	0.77 ± 0.33* $^{\nabla}$	0.66 ± 0.30$^{\nabla\#}$
Insulin (ρmol/L)	144.12 ± 39.84*	160.24 ± 47.45* $^{\nabla}$	128.39 ± 31.78$^{\nabla}$

*** # ∇ = Significant difference within groups ($p \leq 0.05$; non-parametric t-test)**

Table 4: Means and standard deviations of the changes in the plasma and serum biochemistry of some metabolic variables as measured at rest, maximal activity and 30 min of recovery of the sedentary males

Parameters	Fasting	Max. activity	30 min recovery
Haematocrit	46.36 ± 4.52*	50.64 ± 4.27* $^{\nabla}$	46.90 ± 4.59$^{\nabla}$
Albumin (g/L)	48.68 ± 2.42	52.29 ± 3.75	45.43 ± 16.98
Total protein (g/L)	71.71 ± 5.03*	70.31 ± 6.39*	70.56 ± 13.52
Glucose (mmol/L)	5.05 ± 0.28	5.16 ± 0.48	5.05 ± 0.67
Triglyceride (mmol/L)	1.17 ± 0.49	1.13 ± 0.43	1.07 ± 0.39
Insulin (ρmol/L)	167.55 ± 70.03	225.93 ± 54.24	211.28 ± 106.44

*** # ∇ = Significant difference within group ($p \leq 0.05$; non-parametric t-test)**

The results of the sedentary males, which are shown in Table 4, revealed the same trend as that of the active males except for the total protein and triglyceride response. Both these variables showed a slight decrease from rest to maximal activity, which decreased even more after 30 minutes of recovery. The only significant difference were the increase of the haematocrit from fasting to maximal activity and again after recovery together with the increase in the total protein from fasting to maximal activity.

Changes in the fibrin network structure characteristics and haemostatic variables at rest, maximal activity and 30 minutes of recovery in active and sedentary males respectively

The percentage changes that occurred are illustrated in Figure 1 for the active males and Figure 2 for the sedentary males. In the active males (Table 5), fibrin monomer (FM) had a significant decrease from 15.51 ± 7.04 mg/L at rest to 9.84 ± 2.93 mg/L at maximal activity. D-dimer increased significantly from 10.49 ± 2.26 μg/L to 12.42 ± 3.03 μg/L. C-reactive protein had a non-significant decrease of 0.12 μg/L. Factor VII did not change significantly from rest (70.53 ± 29.88%) to maximal activity (69.42 ± 30.77%), but decreased to 63.43 ± 29.03% during recovery, that was significantly lower than the resting value. The response of fibrinogen was non-significant although there was a decrease from fasting to maximal activity, which returned to resting values 30 minutes after maximal activity. The permeability coefficient increased non-significantly from rest (15.46 ± 9.07 $cm^2 \times 10^{11}$) to maximal activity (17.27 ± 9.72 $cm^2 \times 10^{11}$) with a slight decrease after 30 minutes of recovery (17.03 ± 6.20 $cm^2 \times 10^{11}$). More pronounced changes were seen in the mass-length ratio where the values decreased from rest (56.31 ± 36.94 daltons/cm $\times 10^{12}$) to maximal activity (43.65 ± 15.09 94 daltons/cm $\times 10^{12}$) and increased after 30 minutes of recovery (80.80 ± 99.72 94 daltons/cm $\times 10^{12}$). These changes were however, not significant.

Table 5: Means and standard deviations of the changes in the fibrin network structure characteristics and some haemostatic variables as measured at rest, maximal activity and 30 min of recovery of the active males (n = 15)

Parameters:	Rest	Max. activity	30 min recovery
Fibrin monomer (mg/L)	15.51 ± 7.04*	9.84 ± 2.93*	-
D-dimer (μg/L)	10.49 ± 2.26*	12.42 ± 3.03*	-
C-reactive protein (mg/L)	3.12 ± 1.18	3.06 ± 1.08	-
Factor VIIc (%)	70.53 ± 29.88#	69.42 ± 30.77	63.43 ± 29.03#
Fibrinogen (g/L)	3.21 ± 1.01	2.86 ± 0.71	3.19 ± 1.06
Permeability coefficient(K_s) ($cm^2 \times 10^{11}$)	15.46 ± 9.07	17.27 ± 9.72	17.03 ± 6.20
Mass-length ratio (μ_T) (daltons/cm $\times 10^{12}$)	56.31 ± 36.94	43.65 ± 15.09	80.80 ± 99.72

* # = Significant difference within group ($p < 0.05$; non-parametric t-test)

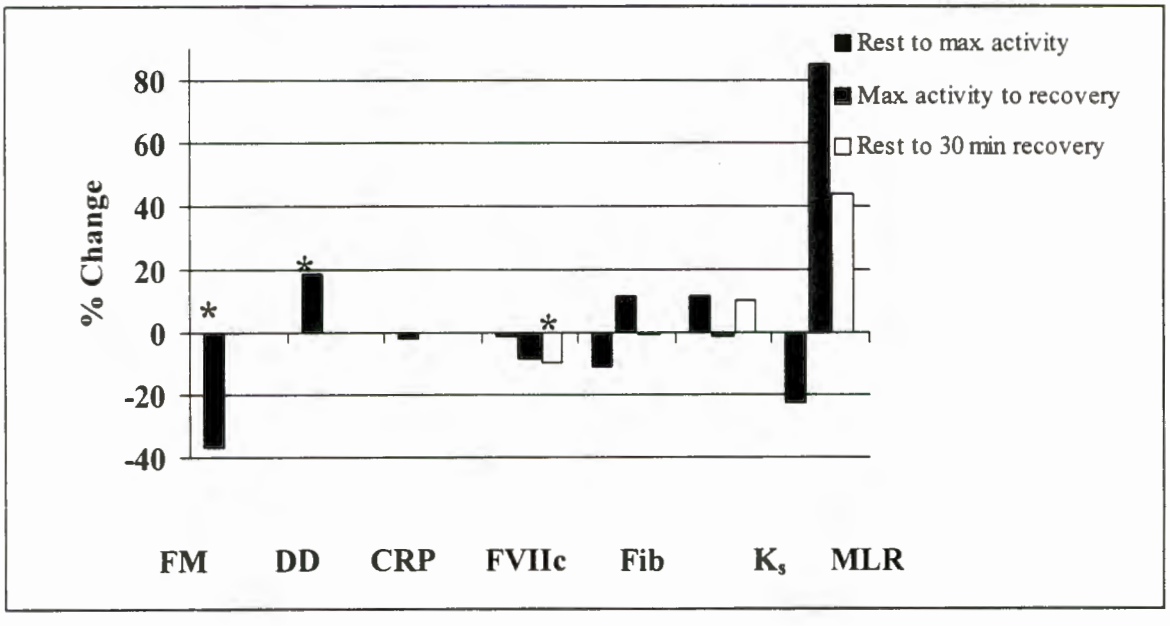

Figure 1 The changes in the fibrin network structure characteristics and haemostatic variables of the active males (n = 15) as a percentage change from rest to maximum activity, maximum activity to 30 min of recovery and rest to 30 minutes of recovery

*** Significantly different ($p \leq 0.05$). FM = fibrin monomer; DD = D-dimer; CRP = C-reactive protein; FVIIc = Factor VIIc; Fib = fibrinogen; K_s = permeability; MLR = mass-length ratio**

The results of the percentage changes in the fibrin network structure characteristics and the haemostatic variables of the sedentary males are given in Figure 2 with the determined values in Table 6. The pattern of response of these sedentary males to maximal activity differed from that of the active group. Fibrin monomer showed a slight increase from 13.25 ± 4.97 mg/L at rest to 14.36 ± 5.06 mg/L during maximal activity. D-dimer had an inverse response where a resting value of 9.36 ± 1.60 μg/L increased to 10.14 ± 2.13 μg/L during maximal activity. A significant decrease in C-reactive protein was obtained with maximal activity from 2.99 ± 0.51 mg/L to 2.77 ± 0.46 mg/L. The changes in Factor VIIc and fibrinogen were not significant.

In the case of the fibrin network structure characteristics, no significant changes occurred, except for the mass-length ratio that had a significant decrease from maximal activity to 30 minutes of recovery. The permeability coefficient decreased from fasting to maximal activity with 2.86%. A further decrease of 11.06% occurred from maximal activity to 30 minutes of recovery. This resulted in a total decrease of 13.92% from initial rest to 30 minutes of rest post-activity. The mass-length ratio increased with 78.79% from rest to maximal activity and decreased with 61.83% from maximal activity to 30 minutes of recovery. This represents a decrease of 31.75% from before exercise values to the resting value after 30 minutes of recovery.

Table 6: Means and standard deviations of the changes in the fibrin network structure characteristics and haemostatic variables as measured at rest, maximal activity and 30 min of recovery of the sedentary males (n = 14)

Parameters:	Rest	Max. activity	30 min recovery
Fibrin monomer (mg/L)	13.25 ± 4.97	14.36 ± 5.06	-
D-dimer (μg/L)	9.36 ± 1.60	10.14 ± 2.13	-
C-reactive protein (mg/L)	2.99 ± 0.51*	2.77 ± 0.46*	-
Factor VIIc (%)	43.09 ± 26.64	45.74 ± 28.97	51.96 ± 40.81
Fibrinogen (g/L)	2.47 ± 0.80	2.77 ± 1.19	2.72 ± 0.94
Permeability coefficient (K_s) ($cm^2 \times 10^{11}$)	23.46 ± 8.22	22.79 ± 9.31	20.27 ± 9.35
Mass-length ratio (μ_T) (daltons/cm $\times 10^{12}$)	70.68 ± 54.66	126.37 ± 97.53$^{\nabla}$	48.24 ± 26.55$^{\nabla}$

*** ∇ = Significant difference within group ($p \leq 0.05$; non-parametric t-test)**

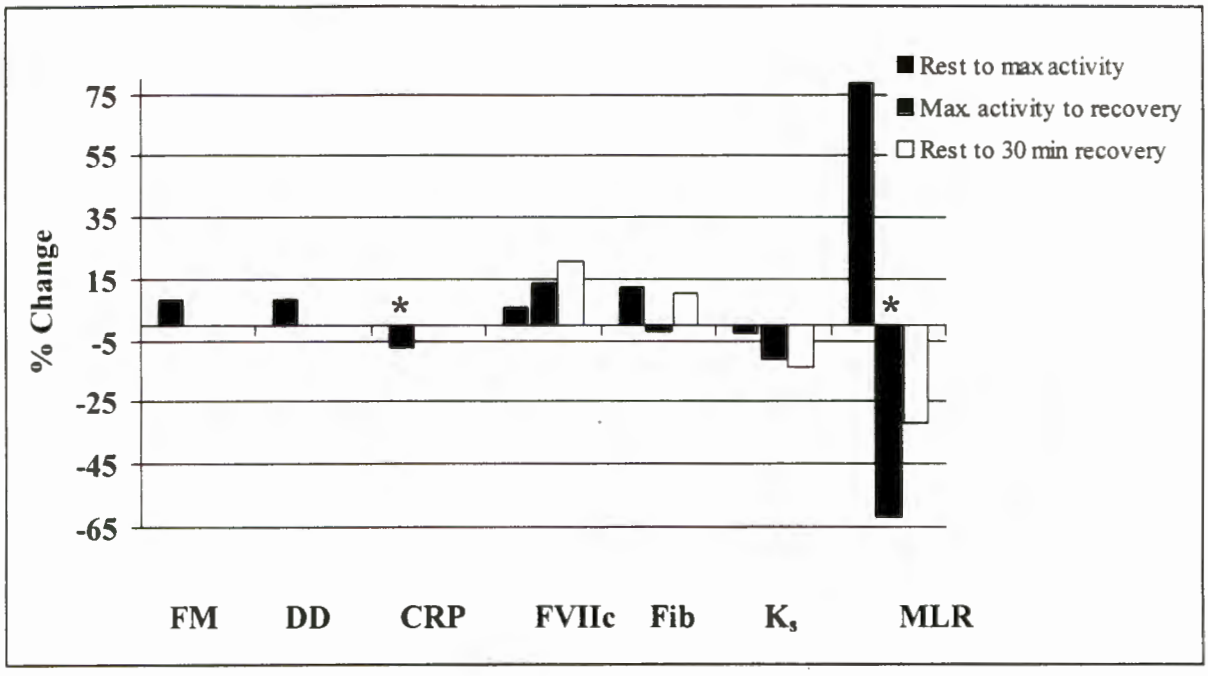

Figure 2 **The changes in the fibrin network structure characteristics and haemostatic variables of the sedentary males (n = 14) as a percentage change from rest to maximum activity, maximum activity to 30 min of recovery and rest to 30 minutes of recovery**

*** Significantly different ($p \leq 0.05$). FM = fibrin monomer; DD = D-dimer; CRP = C-reactive protein; FVIIc = Factor VIIc; Fib = fibrinogen; K_s = permeability; MLR = mass-length ratio**

Discussion

Differences in the values of the fibrin network structure characteristics and the haemostatic variables may give an indication of the effect of training on the parameters. Unfortunately the groups were not completely matched, which made it impossible to compare the results. The differences that existed between the active and sedentary group, is suspected to be the result that long-term physical training has on the body.

Fibrin network structure has been shown to be sensitive to a number of factors including pH, ionic strength, proteins and disease states like peripheral vascular disease (PVD), hypercholesterolaemia [27], diabetes [5] and coronary heart disease [6]. The influence of physical activity on the fibrin network structure has not been previously investigated. Observations from this study show than fibrin network structure architecture may be

changed by means of physical activity. The present study, although limited in numbers of participating subjects and restricted to young healthy men, gives an indication of changes that occur in the fibrin network structure during an one-off maximal exercise session as well as to changes during recovery.

The results of the plasma and serum biochemical analysis for both the sedentary and active males followed the same trends. The active group had more pronounced differences from rest to maximal physical activity and to 30 minutes of recovery after activity than the sedentary group. The most important findings of this study are the changes that occurred in the fibrin network structure characteristics and the haemostatic variables.

The significant decrease in fibrin monomers during exercise may be indicative of an enhanced conversion of the fibrin monomer into the fibrin network structure without increases in thrombin generation. This might have been the reason for the formation of thin, high tensile fibrin network structures that are resistant to lysis. The permeability coefficient that slightly increased with maximal activity, although non-significant, indicates a trend towards increased porosity of the fibrin network structure.

The significant increase in D-dimer, a split product of cross-linked fibrin a marker of fibrin degradation, was also observed during short term maximal exercise [28], a triathlon race [29], long-distance running [30] and marathon running [28]. Prisco *et al.* [31] reported a 215% increase in D-dimer that returned to the baseline values after 48h of recovery. In the light of this evidence, it can be assumed that vigorous exercise results in hyperfibrinolysis *in vivo*, reflected in increased D-dimer formation.

The results indicate an inclination for the blood of the active males to form fibrin network structures that are more rigid, although slightly more porous during maximal activity when compared to the fibrin network structure formed during rest. After 30 minutes of recovery, MLR increased, non-significantly, to values higher than the initial MLR before maximal activity. This may indicate that the fibrin network structure after 30 minutes formed in active males, tend to be less detrimental, in that more porous and less rigid fibrin network structures are formed that are associated with the formation of less detrimental blood clots. These improved networks indicate a possible long-term beneficial effect of exercise or

regular activity on haemostasis, which could be related to effects on fibrinolytic potential. However, more studies on larger groups are necessary to confirm these observations.

The results obtained from the sedentary males indicated that coagulation increased during maximal physical activity as fibrinogen and Factor VII concentration increased, although non-significantly. The permeability changes indicated a slight decrease in the porosity of the fibrin network structure. The most pronounced effect of the maximal activity was in the change of the MLR. An increase in the MLR indicates that the fibrin fibres were formed by enhanced lateral polymerisation. Networks with fibres of increased thickness are less resistant to lysis [32]. These fibrin network structures were found during maximal activity, with a significant decrease in the MLR after 30 minutes of recovery. The decrease in the MLR should result in thin fibres that are less permeable and more resistant to lysis. The total result of maximal exercise in sedentary males indicated that fibrin network structures formed after 30 minutes of recovery were more rigid, less porous and possibly more prone to the formation of detrimental blood clots.

The implications of these findings are not clear. Plasma is an aqueous mixture of proteins, lipids, carbohydrates, amino acids, salts and other substances [33]. A change in any of these constituents of plasma would directly be reflected in the characteristics of the fibrin networks structure [8]. Fibrin network characteristics reflect the conditions under which plasma fibrinogen polymerises to form the blood clot [34] and therefore the functional characteristics of fibrinogen. This study gives an indication of the influence that an acute maximal exercise might have on the fibrin network structure. More research is needed for a better understanding of the effect of long-term and acute exercise on the fibrin network structure and how it relates to haemostasis, blood rheology, health and ill-health.

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More permeable fibrin network structures formed in active males in response to maximal exercise after a low glycaemic index pre-exercise meal.

Sarah J. Moss[1], Maides M. Malan[2], Lukas I. Dreyer[1], & Hester H. Vorster[3]

[1]Institute for Biokinetics at the School of Biokinetics, Recreation and Sport Science
[2]The School of Pharmacy
[3]The School of Physiology, Nutrition and Family Ecology
Potchefstroom University for Christian Higher Education
Potchefstroom, South Africa.

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Abstract

A previous study by this group indicated that maximal exercise changes the environment in which fibrin network structures are formed [1]. Different diets lead to changes in the composition of body fluids. The effect of physical activity, combined with a specific pre-exercise meal on the fibrin network structure is not known. The ingestion of a high glycaemic index (GI) meal results in a large variation in glucose and insulin response, while a low GI meal results in a slow release of glucose and less variation in insulin levels. The purpose of this study was to investigate the effect of high and low GI pre-exercise meals on the fibrin network structure characteristics during and after acute exercise. The fibrin network structures formed from plasma of young active and sedentary males were characterised at rest and after ingestion of a high and low GI pre-exercise meal, given randomly to the subjects. Network structures were then characterised 1-hour post-meal, at maximal activity and after 30 min of recovery by permeation and turbidity techniques. In response to maximal activity the fibrin network structures formed in plasma of active males had larger permeability, higher mass-length ratios and lower tensile strengths with the ingestion of a low GI pre-exercise meal compared to a high GI meal. In sedentary subjects fibrin network structures with higher tensile strengths and less permeability were formed with the ingestion of the low GI pre-exercise meal, although the MLR was higher than with the ingestion of the high GI pre-exercise meal. It is concluded that the effects of exercise on fibrin networks formed from plasma of healthy young males are influenced by the glycaemic properties of the pre-exercise meal.

Key words: Fibrin network structure, maximal exercise, glycaemic index, males, pre-exercise meal

Introduction

The knowledge of the structure of fibrinogen and of the properties of fibrin polymers that are formed on activation of fibrinogen by thrombin or other enzymes has expanded considerably in the past decade [2,3]. It has been hypothesised that the quality of fibrin networks may be a risk factor for the development of coronary heart disease (CHD) [4].

Fatah *et al.* [5] found that a proneness to formation of tight and rigid fibrin networks with abnormal architecture *in vitro* is associated *in vivo* with myocardial infarction at a young age. Nair *et al.* [4] indicated that abnormal fibrin network structures are associated with diabetes mellitus, a major risk factor for CHD. The fibre thickness and permeability were reduced while the compaction remained relatively unaltered in their diabetic subjects. It has also been established that fibrin network structure is altered in peripheral vascular disease (PVD) and hypercholesterolaemia [6]. Lifestyle changes that have been positively linked to a reduction in mortality and morbidity due to these diseases, are increased physical activity and a prudent diet.

Studies on the influence of acute physical activity on plasma fibrinogen levels have yielded mixed results. Plasma fibrinogen levels have been noted to increase, decrease or remain unchanged [7]. In cross-sectional studies results were less controversial. Most studies indicated that regular physical activity is associated with decreased plasma fibrinogen [7]. A hypercoagulable state is observed after acute exercise [8], but also with an increase in fibrinolysis. Results have been inconclusive with regards to physical training, and further research is needed [9]. The only study known reporting on the effects of physical activity on the organisation of the fibrin network structure indicated that highly active young males form less tight and rigid fibrin network structures 30 minutes after recovery of a maximal activity than at rest. Under similar circumstances sedentary males formed more tight and rigid fibrin networks [1].

Studies of the effect of diet on the fibrin network structure are limited. Veldman *et al.*, [10,11] showed that pectin supplementation and calcium acetate ingestion influenced network structure of hypercholesterolaemic men. The diet can be adapted by regulating the type of carbohydrate ingested in order to facilitate different glucose and insulin responses

[12, 13]. In 1981 the glycaemic index (GI) was proposed as a way to describe and classify the glycaemic response to foods. The GI can be used to rank foods, based on the post-prandial glycaemic response to the food [14]. The underlying principle of the GI is that carbohydrate in low GI foods is digested and absorbed more slowly than in high GI foods. The slowing of absorption of carbohydrate reduces the immediate post-prandial glycaemic response [15]. Sports men and women are advised to follow high carbohydrate diets [16] and to consume pre-exercise or pre-event meals also high in carbohydrate.

The purpose of this study was to determine the combined effect of a low and high GI pre-exercise meal and physical activity on the characteristics of the fibrin network structure. The influence of these meals on the fibrin network structure characteristics at rest and changes during maximal activity after ingestion was determined as well.

Materials and Methods

Subjects

A total of 29 young, healthy males between the ages of 19 and 30 years and with a body mass index (BMI) of 18 – 25 kg/m^2 were recruited to voluntarily participate in the study which was approved by the Ethics Committee of the University of Potchefstroom for Christian Higher Education. Fifteen of the subjects were highly active (sports men) and 14 were sedentary (participating in no organised physical activity). The active males ran between 30 and 60 km/week and all of them had a best 10 km time trail of less than 40 min during the preceding 3 months. None of the subjects had any chronic disease, smoked or drank alcohol. No medication or supplements were taken 10 days before testing, as recommended by Van den Burg *et al.* [17]. All the participants completed a 24 hour-recall dietary intake questionnaire [18], which was used to determine their energy intake. According to these results, a pre-fasting, low fibre meal was compiled. The subjects were given the meal and asked to abstain from eating for the following 12 hours before testing. They were asked to abstain from physical activity during the previous 24 hours. This pre-fasting meal was to prevent the second meal effect on the response to the pre-exercise

meal [15]. Three of the 14 sedentary males withdrew from the protocol before the maximal activity was performed.

The composition of the pre-exercise glycaemic index meal

The high GI meal was prepared with warm refined maize meal and glucose, containing 50g of carbohydrate. The low GI meal was prepared with cooked samp that was allowed to cool and served with glucose, also containing 50g of carbohydrate. The GI of the high GI meal was 65% and that of the low GI meal 40%. The two meals were randomly allocated to the participants.

Blood sampling

Venous blood samples were taken between 7:30 and 10:00 am as recommended by Szymanski *et al.* [19] to accommodate the changes in plasma fibrinogen levels during the day. Blood was collected without stasis using atraumatic venepuncture by means of a sterile butterfly infusion set into plastic syringes with 1 part 3.8% tri-sodium citrate and 9 parts of blood with 35 µl of 10 000 KIU/ml Trasylol® /aprotinin (Bayer, Germany) [4]. The subjects fasted overnight (12h) to avoid interference of turbidity measurement by abnormally elevated plasma lipids. Plasma was immediately prepared by centrifugation at 3660 rpm for 15 min at room temperature to obtain platelet-poor plasma, which was again centrifuged for 10 min to obtain essential platelet-free plasma. The plasma was dispensed into plastic tubes and stored at -80°C for less than 3 months until the fibrin network characterisation experiments were performed.

Maximal exercise procedure

Twenty millilitres of blood was drawn without stasis upon the arrival of the subjects. The subjects were then randomly designated either a high or a low GI pre-exercise meal. Sixty minutes after ingestion of the meal, a further 20 millilitres of blood was drawn. The

subjects were then subjected to a maximal exercise session. All participants were subjected to a high and a low glycaemic index pre-exercise meal, one week apart.

The resting blood pressure and heart rate were monitored. The subjects were subjected to maximal exertion on a Monark Bicycle ergometer. The test was started by using a 50-watt resistance at 70 rpm [20]. After every three minutes the heart rate (*Cardio Sport*®) and blood pressure (sphygmomanometer) were monitored in order to increase the resistance by 50 watt every four minutes when a steady state in the heart rate was obtained. The resistance was increased until the maximum age-adapted heart rate [21] was obtained, or until the subjects experienced maximum exertion as expressed by the rate of perceived exertion [22]. The exercise was conducted inside an air-conditioned laboratory with the temperature maintained at 23°C. The third blood sample was drawn within 5 min of reaching the maximum heart rate, or alternatively exhaustion. The fourth and final blood sample was drawn after a 30-min recovery period in a stationary position.

The *Physical Work Capacity* (PWC_{max}) for each individual subject was determined by plotting the heart rate against the resistance (watt) [21]. From this graph the maximum resistance was determined and expressed in relationship to the mass (kg) of the subject. This determination was based on the assumption that a linear relationship exists between the heart rate and the resistance at which activity is performed. The more physically conditioned the subject, the higher the resistance will be before exhaustion is reached.

Network permeation experiment (permeability coefficient, K_s)

The fibrin network structures were formed in a disposable fibrinogen pre-coated cuvette in triplicate after a final concentration of 25 mM $CaCl_2$ and 20 IU thrombin was added to the plasma. The cuvette was closed with a plastic stopper covered with silk. The fibrinogen coating together with the silk was to ensure that networks adhere to the internal surface of the cuvette and do not collapse. The fibrin networks were left to gel for two hours after which Tris-buffer was added to prevent the networks from dehydrating. Percolation of the network structures was performed only after 24 hours to ensure complete polymerisation. The porosity of the fibrin network structures were determined by percolating a buffer

(0.02 M Tris-imidazole, 0.01 M NaCl, pH 7.4) at five different hydrostatic pressures through the gel for a recorded time (seconds). The elutes were collected to determine the volume that was percolated. The permeability coefficient (K_s) was calculated as previously described [23] according to equation 1.

$$K_s = \frac{Q \times L \times \mu}{t \times A \times \Delta P} \quad \text{.....(1)}$$

where Q (in cm^3) is the volume of the liquid having the viscosity μ (in poise.dyne x s/cm^2) flowing through a chamber of the length L (in cm). A is the area (in cm^2) of the chamber and t is the time (in s) for passing of the liquid through the chamber under a pressure gradient P (in $dyne/cm^2$). The CV for the method was 10.74%.

Mass-length ratio determinations (MLR, μ_T)

MLR was determined by the turbidity technique (μ_T) where turbidity was calculated as optical density multiplied by 2.303. The fibrin network structures were formed in disposable cuvettes as for the permeation technique. The turbidity of the networks was recorded with intervals of 2nm over a wavelength range of between 600nm and 800nm (*Shimadzu UV-2100 Visible Recording Spectrometer*) with unclotted plasma in the reference cell. $c/T\lambda^3$ was plotted as a function of the $1/\lambda^2$, where T is the turbidity (2.303 x optical density), λ is the wavelength and c is the concentration of fibrinogen in mg/ml. The intercept A of this plot was used to calculate the average mass-length ratio, μ_T, according to the equation 2 [24]. The CV for the method was 10.20%.

$$\mu_T = \frac{10}{1.48A} \times 10^{12} \text{ daltons/cm} \quad \text{.....(2)}$$

Determination of the compaction of the fibrin network structure

Compaction is one of three techniques used for the characterisation of fibrin network structure. It was introduced by [25]. It is a simple, time economical, and highly reproducible technique that has been correlated with the tensile characteristics of fibrin fibres [4]. Compaction was measured by preparing fibrin networks from pooled, platelet free plasma in 1.5ml eppendorf microcentrifuge tubes, pre-sprayed with a lecithin based aerosol (Spray and Cook®, Colman Foods, South Africa) to render the surface non-adhering. Calcium and thrombin (20 IU thrombin and 25 mM $CaCl_2$) were added to 1ml of plasma and left overnight at room temperature for polymerisation.

The networks were centrifuged at 8000g for 45 seconds in an Eppendorf centrifuge (model 5415C, West Germany). The volume of the fluid expelled from the network was measured with a 1ml syringe and expressed as a percentage of the initial volume of the network [26]. The CV for this method was determined as 7.64%.

Measurement of plasma fibrinogen and Factor VII concentrations

Functional plasma fibrinogen concentration was determined by an adapted Clauss method [27] using an *ACL 200*™ automated coagulation analyser and reagents from Instrumentation Laboratories (IL) (Milan, Italy). Fibrinogen standards and controls were purchased from IL. The CV for the method was 8.24%.

Plasma Factor VII was determined with a one-stage clotting assay on an ACL 200 with a PT-Fib kit from IL (Milan, Italy). The CV for the method was 4.12%.

Haematocrit

EDTA blood was used to determine haematocrit in a haematocrit centrifuge (Hettich Zentrifugen Haematocrit, Tutlingen).

Blood biochemistry

Serum albumin, total protein, triacylglycerol and glucose concentrations were determined with the relevant Boehringer Mannhein kits on a Kinetic reader (Biotec Instruments). A CV of less than 3% was found for all the methods. Insulin levels were determined by radioimmunoassay (with a Packard Cobra Auto Gamma Counter) and had a CV of 25.85%.

Statistical Analysis

All statistical analyses were performed with the SPSS system for Windows® (1999) programme. The means and standard deviations were determined by descriptive statistics. Non-parametric paired t-tests were performed to determine significant differences between fasting, 1-hour post-GI meal, maximal activity and 30 minutes of recovery for the sedentary and active groups respectively. Spearman correlation coefficient was determined between the changes in the serum variables and the changes in the fibrin network structure characteristics. All tests with a p-value of $p \leq 0.05$ were considered statistically significant.

Results

Characteristics of the subjects at rest

The same subjects as in the previous study were used, which resulted in the same basic characteristics for the active and sedentary males as indicated in Table 1 of the previous study [1]. The results indicated that the active and sedentary groups were not matched for their mass, BMI, percentage body fat or resting heart rate. This means that the response of the two groups to a maximal exercise cannot be compared. The results of the active and sedentary groups will thus be discussed separately.

The response of the active males to a maximal exercise test was normal with the heart rate and systolic blood pressure increasing, while the diastolic blood pressure decreased. The

active males, being physically more fit, had a high physical work capacity (PWC_{max})-index due to the high resistance that was exercised at in relation to the body mass (Table 1).

Table 1: Means and standard deviations of the average physiological response of the active and sedentary males to maximal exercise 1 hour after a pre-exercise meal

	Active (n = 15)	Sedentary (n = 11)
Max. activity heart rate (bpm)	189.53 ± 10.27	188.55 ± 6.51
Max. activity systolic BP(mm Hg)	181.40 ± 15.75	188.45 ± 16.60
Max. activity diastolic BP(mm Hg)	63.31 ± 8.80	69.68 ± 9.41
Max. resistance (watt)	303.33 ± 63.24	243.41 ± 33.25
PWC_{max} (watt/kg)	4.53 ± 0.82*	3.13 ± 0.71*

BP = blood pressure; Max. = maximal; PWC_{max} = physical work capacity at age predicted maximum heart rate. * Significant difference between groups ($p \leq 0.05$)

Fasting blood biochemistry variables of the active and sedentary males

The results of the blood biochemistry of the active males at rest are reported in Table 2 for the high GI and Table 3 for the low GI tests. The active males were healthy, with the variables known to be influenced by physical activity in the lower end of the normal range. The values for the liver enzymes were all within the normal range. All the results of this study have taken the plasma volume shift that occurs during maximal activity, into consideration and corrected for the shift with the haematocrit concentration. The reported results are thus after the adaptation for the plasma volume shift. The changes that were observed in the haemostatic and fibrin network structure characteristics are shown in Figure 1 to 5.

Changes in the blood biochemical variables and fibrin network structure characteristics 1 hour after ingesting a high and low GI pre-exercise meal

A significant decrease in haematocrit was obtained 1 hour after ingestion of the high GI meal in the active males (Table 2). When a low GI meal was ingested, the albumin and total protein concentrations decreased significantly (Table 3). With both the high and low GI meals the insulin concentration increased significantly.

Table 2: Changes in the blood biochemistry of active males at fasting, 1hour post-high GI pre-exercise meal, maximal activity, and 30 minutes after recovery

PARAMETERS	ACTIVE (n = 15)			
	Fasting	**1h post meal**	**Max activity**	**30 min of Recovery**
	Mean ± SD	***Mean ± SD***	***Mean ± SD***	***Mean ± SD***
Haematocrit (%)	$43.20 \pm 2.93^{*\#\clubsuit}$	$42.27 \pm 3.58^{*\nabla}$	$44.33 \pm 3.04^{\nabla\blacklozenge\#}$	$42.13 \pm 3.23^{\blacklozenge\clubsuit}$
Albumin (g/L)	$50.93 \pm 2.43^{\#}$	$50.93 \pm 1.58^{\nabla}$	$52.15 \pm 2.74^{\nabla\#}$	51.74 ± 3.08
Total protein (g/L)	$70.80 \pm 3.45^{\#}$	$70.20 \pm 2.62^{\nabla}$	$72.59 \pm 3.39^{\nabla\blacklozenge\#}$	$71.42 \pm 3.42^{\blacklozenge}$
Glucose(mmol/L)	4.67 ± 0.24	4.93 ± 0.77	4.50 ± 0.78	4.71 ± 0.58
Triglyceride (mmol/L)	$0.77 \pm 0.27^{\clubsuit}$	$0.78 \pm 0.29^{\circ}$	$0.76 \pm 0.33^{\blacklozenge}$	$0.62 \pm 0.26^{\blacklozenge\clubsuit\circ}$
Insulin (ρmol/L)	$181.81 \pm 38.10^{*\clubsuit}$	$300.22 \pm 96.04^{*\nabla\circ}$	$226.81 \pm 73.62^{\nabla}$	$234.66 \pm 78.59^{\clubsuit\circ}$

GI = glycaemic index

*** # ° ∇ ♣ ♦ = Significant difference ($p \leq 0.05$) within groups**

Table 3: Changes in the blood biochemistry of active males at fasting, 1hour post-low GI pre-exercise meal, maximal activity and 30 minutes after recovery

PARAMETERS	ACTIVE (n = 15)			
	Fasting	**1h post meal**	**Max activity**	**30 min of Recovery**
	Mean ± SD	***Mean ± SD***	***Mean ± SD***	***Mean ± SD***
Haematocrit (%)	$42.43 \pm 2.80^{\#}$	$42.30 \pm 3.66^{\nabla}$	$43.97 \pm 4.08^{\nabla\blacklozenge\#}$	$41.90 \pm 4.17^{\blacklozenge}$
Albumin (g/L)	$49.13 \pm 1.96^{*\clubsuit}$	$46.47 \pm 1.13^{*\nabla\circ}$	$48.33 \pm 1.85^{\nabla\blacklozenge}$	$50.80 \pm 2.56^{\blacklozenge\clubsuit\circ}$
Total protein (g/L)	$68.73 \pm 3.15^{*\clubsuit}$	$65.67 \pm 2.26^{*\nabla\circ}$	$67.99 \pm 3.36^{\nabla\blacklozenge}$	$72.31 \pm 4.36^{\blacklozenge\clubsuit\circ}$
Glucose(mmol/L)	4.47 ± 0.40	4.15 ± 1.30	4.48 ± 0.69	4.70 ± 0.35
Triglyceride (mmol/L)	0.89 ± 0.40	$0.82 \pm 0.34^{\nabla\circ}$	$0.87 \pm 0.39^{\nabla\blacklozenge}$	$0.77 \pm 0.32^{\blacklozenge\circ}$
Insulin (ρmol/L)	$141.38 \pm 33.81^{*\#}$	$206.63 \pm 52.35^{*\circ}$	$186.73 \pm 67.08^{\blacklozenge\#}$	$144.28 \pm 44.03^{\blacklozenge\circ}$

GI = glycaemic index

 # ♦ ∇ ♣ ° = Significant difference ($p \leq 0.05$) within groups

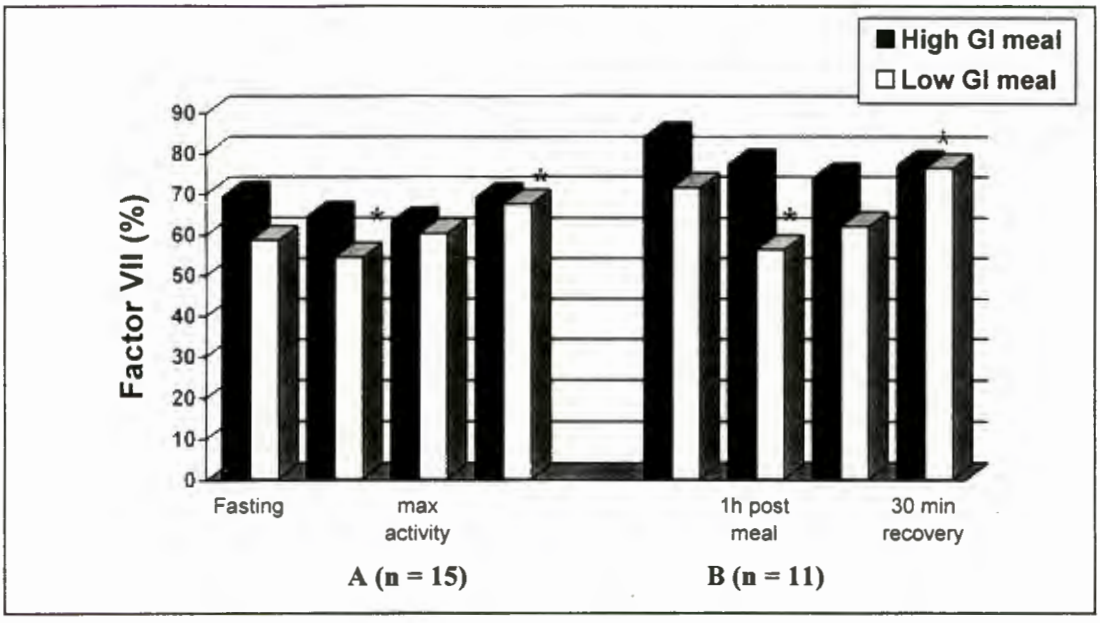

Figure 1 **Changes in Factor VII concentrations of the active (A) and sedentary (B) males obtained at fasting, 1h post-glycaemic index pre-exercise meals, maximal activity and 30 minutes of recovery**

*** Significant difference ($p \leq 0.05$) within groups**

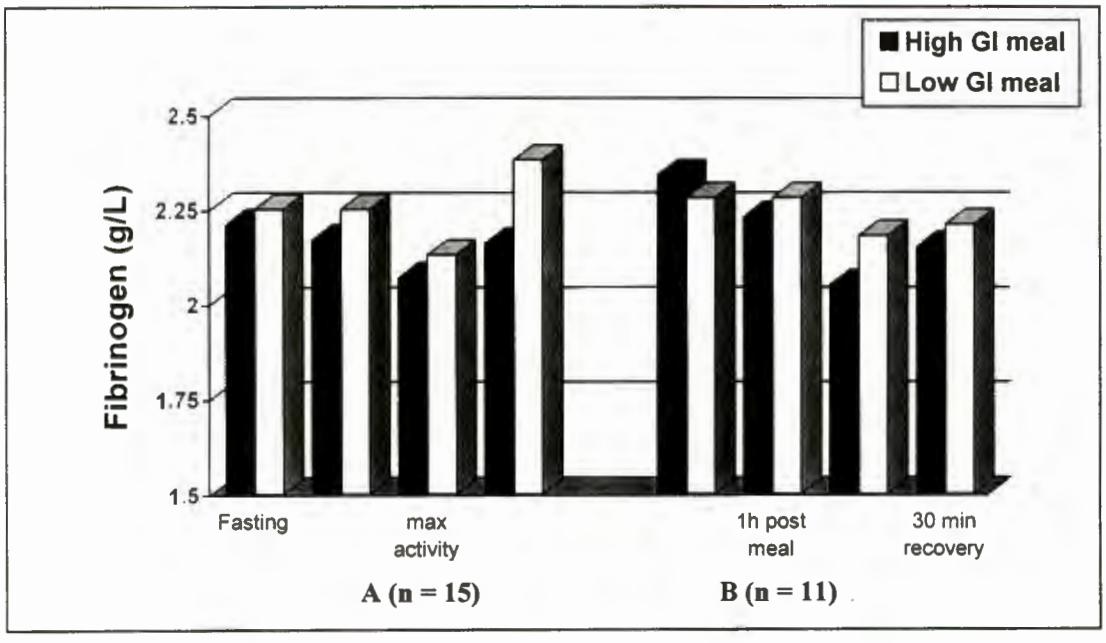

Figure 2 **Changes in fibrinogen of the active (A) and sedentary (B) males obtained at fasting, 1h post-glycaemic index pre-exercise meals, maximal activity and 30 minutes of recovery**

The high GI meal resulted in a 65.13% increase of insulin, supporting the findings of Costill *et al.* [28] and Ahlborg & Bjorkman [29]. The low GI meal increased insulin with 46.17%. These findings are consistent with the findings of De Marco *et al.* [30] and Jenkins *et al.* [31]. When the response of the serum variables to the low and high GI meals are compared, glucose tends to increase with the high GI meal and decrease with the low GI meal.

The only significant change in fibrin network structure characteristics, was an increase in the MLR with ingestion of a high GI meal (Table 6). The high GI meal tended to decrease compaction, and a low GI meal resulted in a slight elevation.

The blood biochemistry results from the sedentary males obtained 1-hour post-GI meal only indicated a significant increase in insulin (Tables 4 and 5). The response of the high GI meal resulted in a 12% larger insulin response than the low GI meal. The rest of the variables responded in a similar fashion with both GI meals. Although no significant changes in the fibrin network structures were found, an increase in MLR was observed with the low GI meal and a decrease in MLR with the high GI meal (Table 7).

Table 4: Changes in the blood biochemistry of the sedentary males at fasting, 1 hour post-high GI pre-exercise meal, maximal activity, and 30 minutes after recovery

PARAMETERS	SEDENTARY (n = 11)			
	Fasting	**1h post meal**	**Max activity**	**30 min of Recovery**
	Mean ± SD	***Mean ± SD***	***Mean ± SD***	***Mean ± SD***
Haematocrit (%)	45.18 ± 3.03$^{\#}$	45.09 ± 2.51$^{\nabla}$	47.45± 3.27$^{\nabla\blacklozenge\#}$	44.91 ± 2.70$^{\blacklozenge}$
Albumin (g/L)	50.91 ± 1.97$^{\#}$	50.27 ± 1.74$^{\nabla\circ}$	51.99 ± 2.00$^{\nabla\#}$	51.36 ± 1.81°
Total protein (g/L)	70.73 ± 4.05	69.82 ± 3.52$^{\nabla\circ}$	71.85 ± 3.81$^{\nabla}$	71.13 ± 3.42°
Glucose(mmol/L)	4.99 ± 0.35$^{\#}$	5.63 ± 1.45$^{\nabla}$	4.20 ± 0.67$^{\nabla\#}$	4.75 ± 0.86
Triglyceride (mmol/L)	1.17 ± 0.74	1.22 ± 0.65°	1.18 ± 0.64$^{\blacklozenge}$	0.92 ± 0.45$^{\blacklozenge\circ}$
Insulin (ρmol/L)	236.12 ± 68.71*	431.67±248.69*$^{\circ}$	247.89 ± 97.49	257.60 ± 108.77°

GI = glycaemic index

 # ° ∇ ♣ ♦ = Significant difference ($p \leq 0.05$) within groups

Table 5: Changes in the blood biochemistry of sedentary males at fasting, 1 hour post-low GI pre-exercise meal, maximal activity and 30 minutes after recovery

PARAMETERS	SEDENTARY (n = 11)			
	Fasting	**1h post meal**	**Max activity**	**30 min of Recovery**
	Mean ± SD	***Mean ± SD***	***Mean ± SD***	***Mean ± SD***
Haematocrit (%)	45.36 ± 2.72$^{\#}$	45.32 ± 2.19$^{\nabla}$	47.45 ± 3.45$^{\nabla\blacklozenge\#}$	45.55 ± 2.54$^{\blacklozenge}$
Albumin (g/L)	49.09 ± 1.81$^{\blacklozenge}$	48.82 ± 1.94	50.73 ± 2.72	50.34 ± 2.29$^{\blacklozenge}$
Total protein (g/L)	68.82 ± 3.68$^{\#\blacklozenge}$	68.45 ± 4.27$^{\nabla}$	71.65 ± 5.04$^{\nabla\#}$	70.76 ± 4.75$^{\blacklozenge}$
Glucose(mmol/L)	4.52 ± 0.35	4.63 ± 0.97	4.49 ± 0.84	4.53 ± 0.54
Triglyceride (mmol/L)	1.01 ± 0.44	1.02 ± 0.40°	1.05 ± 0.41$^{\blacklozenge}$	0.92 ± 0.37$^{\blacklozenge}$°
Insulin (ρmol/L)	170.01±35.73*$^{\#\blacklozenge}$	290.44±128.59*°	236.12±60.10$^{\blacklozenge\#}$	155.91± 35.99$^{\blacklozenge\blacklozenge}$°

GI = glycaemic index

 # ♦ ∇ ♣ ° = Significant difference ($p \leq 0.05$) within groups

Changes in the blood biochemical variables and fibrin network structure characteristics in response to maximal exercise after ingesting a pre-exercise high and low GI meal

The haematocrit, albumin and total protein of the active males increased significantly with maximal exercise after the high and low GI meal. A significant decrease in insulin was found with the high GI meal (Table 2), while the triglyceride concentration decreased significantly with the low GI meal (Table 3). The indication from literature is that a single bout of exercise has the potential to induce short term, transient changes in the lipids. In men a decrease in triglycerides was observed [32]. The response of the fibrinogen concentration to the high and low GI meals in combination with maximal exercise was not significant. This is in agreement with other authors [33, 34]. Results of the fibrin network structure indicated a significant increase in compaction and MLR with the ingestion of the low GI meal (Table 6). Compaction as a characteristic of the fibrin network structure has been correlated with the tensile characteristics of the fibrin fibres [26]. The increase in MLR is an indication of the fibrin fibres becoming shorter and thicker. These networks are more porous and less resistant to lysis. These characteristics are synonymous with less detrimental fibrin network structures [35].

Table 6: Changes in the haemostatic variables and fibrin network structure characteristics of the active males at fasting, 1 hour post-high and low GI meal, maximal activity and 30 minutes after recovery

PARAMETERS	ACTIVE (n = 15) (HGI)				ACTIVE (n = 15) (LGI)			
	Fasting	1h post meal	Max activity	30 min recovery	Fasting	1h post meal	Max activity	30 min recovery
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*
Factor VIIc (%)	69.20 ± 23.87	64.33 ± 26.49	62.99 ± 26.36	68.92 ± 24.98	58.40 ± 17.41	54.20 ± 20.58°	60.09 ± 37.54	67.33 ± 29.48°
Compaction (%)	84.48 ± 9.33	80.73 ± 12.19	84.30 ± 7.43	84.16 ± 4.46	73.88 ± 10.93$^{\#}$	79.96 ± 9.68$^{\nabla}$	85.29 ± 7.03$^{\nabla\blacklozenge\#}$	75.27 ± 12.34$^{\blacklozenge}$
Fibrinogen (g/L)	2.21 ± 0.41	2.17 ± 0.40	2.07 ± 0.43	2.16 ± 0.32	2.25 ± 0.49	2.25 ± 0.49	2.13 ± 0.63	2.38 ± 0.62
Permeability ($cm^2 \times 10^{11}$)	16.79 ± 5.38	16.91 ± 4.13	13.34 ± 4.74	15.56 ± 4.32	17.06 ± 8.79	19.03 ± 8.40	20.49 ± 9.50	18.04 ± 6.86
Mass-length tatio (dalton/$cm^2 \times 10^{12}$)	31.86 ± 8.01$^{*\#\clubsuit}$	38.57 ± 8.15*	42.68 ± 7.82$^{\#}$	41.00 ± 8.75$^{\clubsuit}$	37.09 ± 12.04$^{\#\clubsuit}$	41.59 ± 10.93$^{\nabla}$	65.62 ± 41.65$^{\nabla\#}$	49.56 ± 19.44$^{\clubsuit\circ}$

*** # ° ∇ ♣ ♦ = Significant difference ($p \leq 0.05$) within groups**
HGI = high glycaemic index meal
LGI = low glycaemic index meal

Table 7: Changes in the haemostatic variables and fibrin network structure characteristics of the sedentary males at fasting, 1 hour post-high and low GI pre-exercise meal, maximal activity and 30 minutes after recovery

PARAMETERS	SEDENTARY (n = 15) (HGI)				SEDENTARY (n = 11) (LGI)			
	Fasting	1h post meal	Max activity	30 min recovery	Fasting	1h post meal	Max activity	30 min recovery
	Mean ± SD	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*	*Mean ± SD*
Factor VIIc (%)	83.91 ± 30.95	77.18 ± 30.62	73.77 ± 33.54	76.91 ± 18.93	71.36 ± 25.05	56.10 ± 28.82°	61.78 ± 32.50	76.10 ± 21.46°
Compaction (%)	89.51 ± 5.62♣	87.83 ± 5.88	87.17 ± 4.17	83.16 ± 6.55♣	78.54 ± 10.47	76.95 ± 10.53	79.46 ± 6.50	79.33 ± 10.69
Fibrinogen (g/L)	2.34 ± 0.46	2.23 ± 0.52	2.05 ± 0.39	2.15 ± 0.40	2.28 ± 0.49	2.28 ± 0.49	2.18 ± 0.43	2.21 ± 0.58
Permeability ($cm^2 \times 10^{11}$)	26.79 ± 20.25	23.82 ± 15.94	21.20 ± 9.05	20.35 ± 8.30	21.23 ± 17.66	19.24 ± 12.64	18.58 ± 6.54	21.83 ± 9.64
Mass-length ratio (dalton/$cm^2 \times 10^{12}$)	75.60 ± 67.36	48.36 ± 16.66	60.61 ± 29.40	57.14 ± 26.76	41.57 ± 11.16♣	46.54 ± 10.60°	84.84 ± 103.20	60.90 ± 17.22♣°

° ♣ = Significant difference ($p \leq 0.05$) within groups
HGI = high glycaemic index meal
LGI = low glycaemic index meal

Although the changes were not significant, the high GI meal in combination with a maximal exercise resulted in a slight decrease in glucose, FVIIc and K_s. The same variables increased with the low GI meal. This corresponds with a 7.5% decrease in FVIIc obtained with a 4.8 km run by healthy adults in approximately 18 minutes [36].

When the sedentary males were subjected to maximal exercise, a significant increase in haematocrit, albumin and total protein was observed after both the high GI and low GI meal (Table 4 and 5). Glucose decreased significantly when the high GI meal was taken. Although there were no significant changes in the fibrin network structure (Table 7), the compaction with the low GI meal was lower than with the high GI meal.

The changes in the blood biochemical variables and fibrin network structure characteristics in response to 30 minutes of recovery from a maximal exercise after ingestion of high and low GI pre-exercise meals

After maximal exercise the subjects were asked to remain in the upright position for 30 minutes of recovery. Haematocrit and triglycerides decreased significantly when the high and low GI meals were taken (Table 2 and 3). This finding supports the results of a 30-minute exercise performed at 75% of maximal heart rate on a bicycle ergometer [37]. After 30 minutes of recovery the active males had a slightly higher glucose concentration on the low GI meal 1 hour after the meal, while the glucose value was lower after recovery when the high GI meal was taken. These results support the findings of 6 cyclists ingesting a low GI pre-exercise meal [38]. Significant increases were found after maximal exercise and the ingestion of the low GI meal in albumin and total protein. The only fibrin network structure characteristic that changed significantly was the decrease in compaction (Table 6). The trend in insulin levels of the active males was a return to pre-exercise levels, more so in the low GI meals than the high GI meals. The K_s in both the high and low GI meals changed towards the pre-exercise levels. The K_s after the low GI meal and activity stabilised at higher values than the high GI meal values.

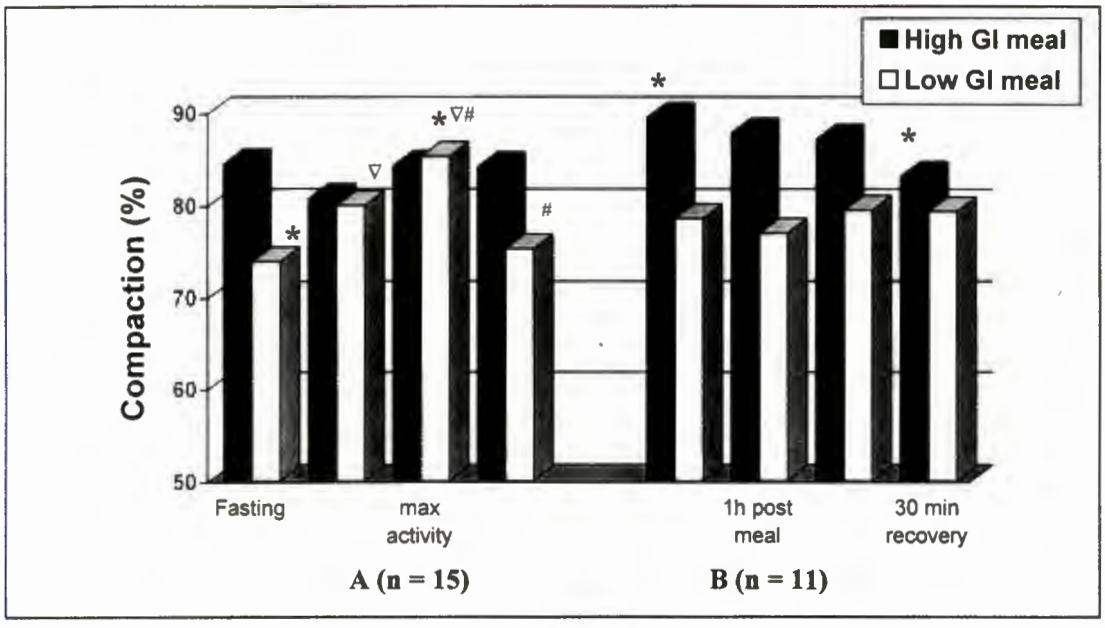

Figure 3 **Changes in compaction of the active (A) and sedentary (B) males obtained at fasting, 1 hour post-glycaemic index pre-exercise meals, maximal activity and 30 minutes of recovery. Same symbols signify significant difference within groups ($p \leq 0.05$)**

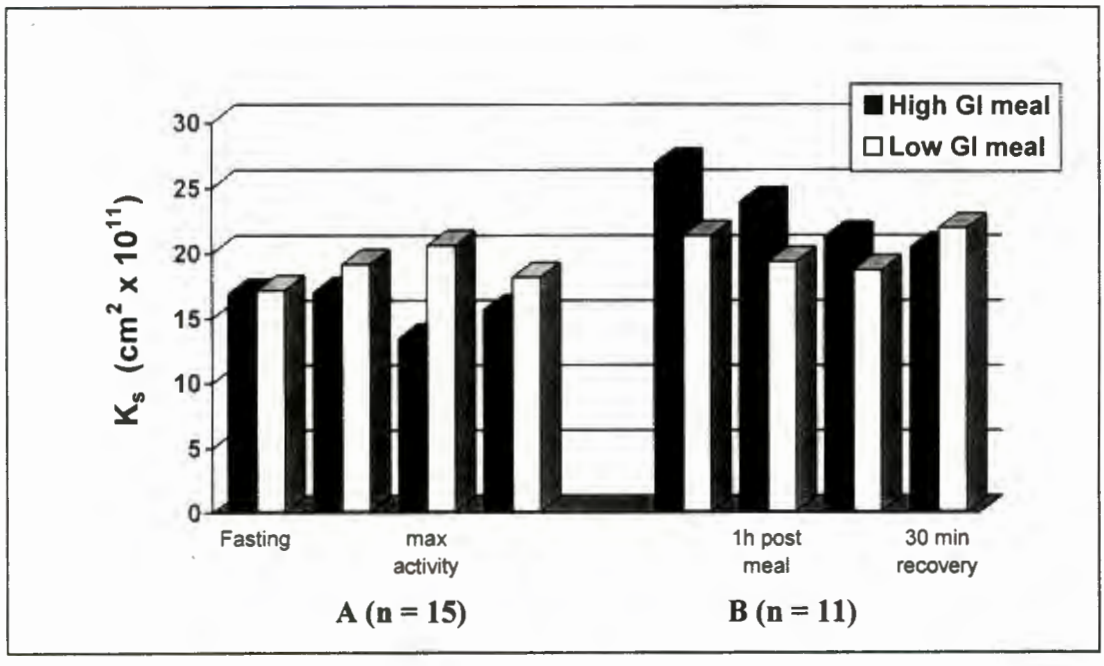

Figure 4 **Changes in permeability coefficient of the active (A) and sedentary (B) males obtained at fasting, 1 hour post-glycaemic index pre-exercise meals, maximal activity and 30 minutes of recovery**

After maximal exercise in combination with a high and low GI meal, the sedentary males showed significant decreases in haematocrit and triglycerides (Tables 4 and 5). Recovery from a maximal exercise after a low GI meal resulted in a significant decrease in insulin. The fibrin network structure showed no significant changes during this stage (Table 7). A trend to an increase in K_s with a decrease in insulin was observed with the low GI meal in combination with recovery from activity. The opposite results were obtained with the high GI meal with regards to insulin and K_s. These results correspond to data obtained from fibrin network structure characteristics of poorly controlled diabetics [4].

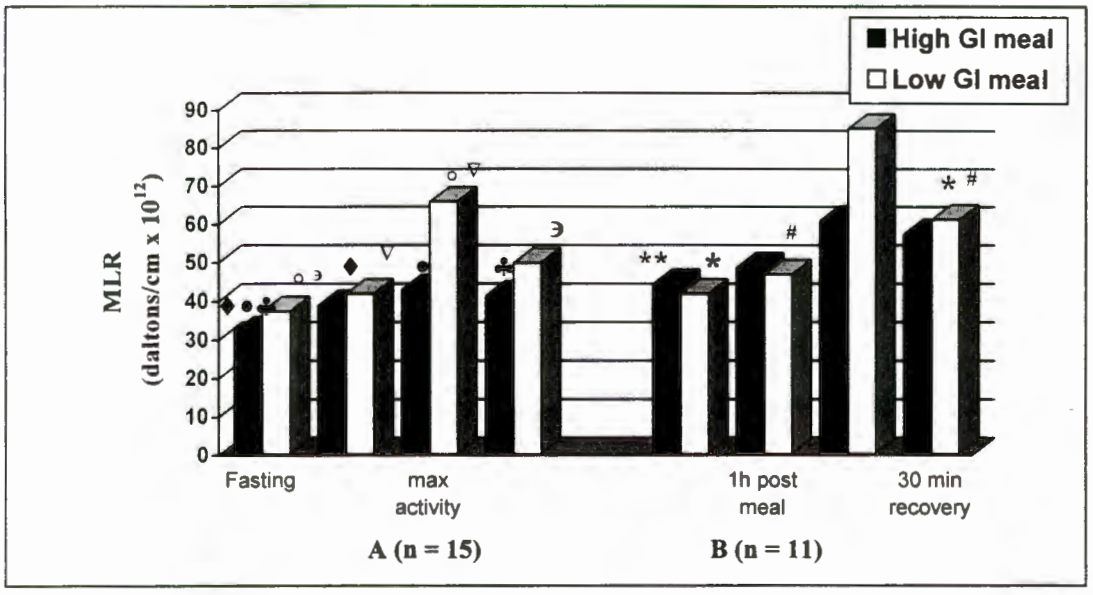

Figure 5: Changes in mass-length ratio (MLR) of the active (A) and sedentary (B) males obtained at fasting, 1h post-glycaemic index pre-exercise meals, maximal activity and 30 minutes of recovery
Similar symbols signify significant differences within groups ($p \leq 0.05$)
**** Value was 75.60 ± 67.36 as a result of 2 outlaying values**

Discussion

The purpose of this study was firstly to determine the influence of the GI of the pre-exercise meal on the fibrin network structure characteristics of physically conditioned and healthy young sedentary males. Secondly, the changes in the fibrin network structure characteristics in response to maximal activity after respectively high and low GI pre-

exercise meals were determined. As the high and low GI meals lead to differences in circulating insulin and glucose responses [39, 14], changes in the characteristics of the fibrin network structure were expected.

The results of this study showed that the sedentary group was not a matched control for the active group. This is due to the fact that the differences that exist between the active and sedentary group, are due to the long-term influence that physical activity brings about. The active group was physically significantly more conditioned than the sedentary males. This difference in physical conditioning of the body would inevitably trigger different response under the same circumstances from the two groups. It is possible that the sedentary males have not established mechanisms to cope with regular bouts of stress in the form of physical activity, and differences in response to meals and exercise can be expected.

In the active males the low GI meal resulted in a positive effect 1-hour post-meal on the blood biochemistry due to the smaller insulin response. Plasma variables are considered to be modulating factors [40] that influence the environment where the fibrin network structures are formed. The increase in compaction indicates a network with lower tensile strength [26]. The response of the sedentary group for the same period indicated that the low GI meal had a positive effect on the biochemical variables and fibrin network structure with the smaller response in insulin together with the increase in the MLR. The increased MLR indicates that the low GI meal might result in shorter and thicker fibrin networks forming. Blombäck [40] has indicated that these networks are less resistant to lysis and thus associated with a lower risk for CVD.

A comparison of the response of the active and sedentary males to the high and low GI meals indicates that the active males had better mechanisms to cope with the high GI meal than the sedentary males. The smaller insulin response and changes in MLR support this statement. In the case of the low GI meal the active males had improved K_s and compaction. Results obtained by Van Gelder *et al.* [41] indicated that permeability (K_s) was not affected by fibre thickness but by the arrangement of the fibrin fibres in the fibrin network.

With the maximal exercise after the ingestion of the high and low GI meals, the results of the active males indicated that consuming a low GI pre-exercise meal would probably be beneficial for fibrin networks. This is due to the low GI meal increasing the MLR and compaction significantly, with a slight increase in the porosity. The results for the sedentary group are not as clear. Spearman's correlation coefficients between the changes in the biochemical variable and the changes in the fibrin network structure characteristics showed that 15% of the variables correlated significantly ($r \geq 0.5$; $p \leq 0.05$). The changes in the fibrin network structure thus correlated with changes in the metabolic environment.

Differences in response to the high GI meal of the two groups are difficult to interpret and further research is needed. The ingestion of a low GI meal led to positive changes in the active males in response to maximal exercise by means of an increased compaction and MLR.

The results during recovery from the maximal exercise for active and sedentary males indicated the possible advantage of the low GI meal (*via* the increase in permeability). The ingestion of a high GI meal before a maximal exercise would only have a positive effect on the fibrin network structure if the person were active since an increase in permeability can be expected.

To conclude, these results showed that the metabolic environment changes after meals and exercise had a direct influence on the formation and architecture of the fibrin network structure. The ingestion of a low GI pre-exercise meal had a "positive" effect on the metabolic environment and fibrin network structure 1-hour after ingestion, after maximal exercise and after 30 minutes of recovery compared to a high GI meal. This "positive" effect was characterised by a smaller response in insulin, and increases in compaction, MLR and permeability. This gives an indication of the formation of fibrin network structures that are shorter, thicker and more porous. These networks should also have less minor networks, resulting in lower tensile strengths due to the increased compaction.

It is suggested that further investigation is required into the influence of other pre-exercise meals on the fibrin network structure during other types of activity, in larger groups of different subjects. Determination of markers of fibrinolysis could result in a better

understanding of the results. The health consequences and possible benefits of the observed effects of the low GI meal compared to the high GI meal before exercise need further investigation.

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CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

To conclude the study on the characteristics of the fibrin network structure, this chapter will discuss the results obtained with the objectives of this study in mind, and draw conclusions on the influence of maximal activity on fibrin network structure characteristics in sedentary and active males. Thereafter changes in these characteristics after a pre-exercise meal intervention will be discussed together with the influence of maximal exercise on fibrin network structure after the ingestion of a high and low GI meal.

This chapter will also consider the limitations of the study and the influence these might have had on the results. Finally, directions for future research concerning physical activity, fibrin network structure characteristics and diet interventions will be recommended with some possible practical implications.

6.2 Changes in the characteristics of the fibrin network structure in active and sedentary males in response to physical activity

6.2.1 Introduction

Several studies have indicated that mortality and morbidity are reduced with increased physical activity (Paffenbarger *et al.,* 1993). The mechanisms through which the benefits of physical activity are achieved have not been fully established. It has been hypothesised that mortality and morbidity might be reduced through changes in the haemostatic balance

(Connelly *et al.*, 1992). The main objective of this study was to determine whether physical activity changes the fibrin network structure characteristics as a possible mechanism for the beneficial effect of physical activity. To investigate this hypothesis the study was conducted with the objectives mentioned in Chapter 1. The extent to which these objectives have been reached will now be discussed.

6.2.2 Changes in metabolic and haemostatic variables in response to maximal exercise and 30 minutes of recovery in active and sedentary males

The metabolic variables that were determined in the study included haematocrit, albumin, total protein, glucose, insulin and triglycerides. With maximal exercise the haematocrit, albumin and glucose increased slightly with insulin levels of the active males reaching significance. Total protein and triglycerides showed a significant decrease with maximal exercise. After 30 minutes of recovery all the variables that increased with maximal activity decreased to fasting levels, except for trigylcerides that showed a further significant decrease. The total protein concentration increased to fasting levels after the 30 minutes of recovery.

The metabolic variables of the sedentary males responded in a similar fashion as in the active males. The haematocrit, albumin, glucose and insulin increased with maximal activity while total protein and triglyceride concentrations decreased. The results obtained after the 30 minutes of recovery also showed that the sedentary males responded in the same fashion as the active males. The haematocrit, albumin, glucose and insulin concentrations decreased to initial fasting levels. The total protein concentration remained at the concentration measured at maximal activity, while serum triglycerides decreased.

The haemostatic variables determined in this study included D-dimer, fibrin monomer, factor VII (FVII) and fibrinogen. With maximal exercise the haemostatic balance of the physical active males moved towards fibrinolysis with the increase of D-dimer and decreases in fibrin monomer and FVII. D-dimer provides information on *in vivo* fibrinolysis (Herren *et al.*, 1994) while increased fibrin monomer and FVIIc are indicative of an increased coagulation potential (Miller, 1993). These changes were not seen to the

same extent in the sedentary males, which may be interpreted as a beneficial effect of physical fitness on haemostasis.

6.2.3 Changes in the fibrin network structure characteristics in response to maximal exercise and 30 minutes of recovery in active and sedentary males

The fibrin network characteristics that were investigated in the study included the permeability coefficient (K_s) and mass-length ratio (MLR). The K_s gives an indication of the porosity of the fibrin network structure while the MLR indicates thrombogenicity potential (Collett *et al.*, 1993). Maximal activity in the case of the active males resulted in increased K_s with a slight decrease in the MLR. This increase might be due to the changes in the glucose and insulin responses that have been shown to result in increased MLR in diabetics compared to healthy persons (Nair *et al.*, 1991b). Thirty minutes of recovery resulted in the permeability stabilising at the maximal exercise level while the MLR increased to levels that were higher than the initial fasting level. This may be an indication of the young active males forming fibrin network structures that led to less detrimental blood clots being formed after acute maximal exercise.

The fibrin network structure of the sedentary males gave the opposite response to maximal activity. The K_s decreased with maximal exercise and stabilised at the same level after 30 minutes of recovery. The MLR increased with maximal exercise, but decreased significantly to levels lower than those determined at fasting. This means that in sedentary young males, fibrin network structures that may be indicative of detrimental blood clots are formed after 30 minutes of a maximal exercise. This may be interpreted as an increased risk for thrombogenicity in the sedentary young males during acute maximal exercise.

6.2.4 The influence of a high and low GI pre-exercise meal on fibrin network structure characteristics and metabolic variables of active and sedentary males 1 hour after meal ingestion

The variables that were measured before and after the meal intervention, were the haematocrit, albumin, total protein, triglyceride, glucose, and insulin concentrations. These

determinations were taken after 12 hours fasting, 1-hour post-GI meal, at maximal exercise and after 30 minutes of recovery. The response of the active males to the GI meals resulted in both the meals increasing insulin levels significantly. The low GI meal, however, resulted in a smaller increase compared to the high GI meal. The same response was found in the sedentary males. The fibrin network structure showed changes in the active males involving increased compaction with the low GI meal. The sedentary males showed an increase in MLR with the low GI meal compared to the high GI meal. The low GI meal thus seems to lead to the formation of more compact fibrin network structures when compared to high GI meals in active men. The low GI meal in sedentary men tended to lead to the formation of fibrin network structures consisting of short and thick fibres, less resistant to lysis when compared to those formed after the high GI meal.

6.2.5 Changes that occur in the fibrin network structure characteristics and metabolic variables of the active and sedentary males in response to a maximal activity and 30 minutes of recovery after ingestion of a high and low GI pre-exercise meal

The high GI meal ingested before maximal exercise resulted in significant decreased insulin levels while the low GI meal led to significant decreased triglycerides. The fibrin network structure was positively influenced by the low GI meal when compared to the effect of the high GI meal. Significant increases in compaction and MLR were found. With a low GI meal in combination with maximal activity the fibrin network tended to consist of shorter and thicker fibres, with more pores and which is thought to be less resistant to lysis. The changes in compaction indicated lower tensile strengths and less minor networks.

The results obtained from the sedentary males were not as conclusive. The high GI meal led to a significant decrease in the glucose concentration, while the low GI meal increased compaction. More research in this area is needed. The Spearman correlation coefficient between the changes in the metabolic variables and changes in the fibrin network structure characteristics indicated that 15% of all the variables correlated. It therefore seems that changes in the metabolic variables influenced the fibrin network structure.

6.2.6 Changes in the haemostatic variables and fibrin network structure after 30 minutes of recovery from a maximal exercise in combination with a high and low GI pre-exercise meal

When the active subjects recovered from the activity after the low GI meal, a significant decrease in insulin was observed. Although the K_s decreased, the value was still higher than the K_s obtained with the high GI meal. In the sedentary males, the low GI meal in comparison with the high GI meal resulted in decreased insulin and increased K_s. Recovery from the activity with the various ingested GI meals, resulted in less porous fibrin network structures being formed in both the active and sedentary groups. These networks are more permeable.

6.2.7 Summary

In conclusion, the active young males seem to have mechanisms in place favouring fibrinolysis during maximal exercise. Adaptations in the internal environment are complementing the formation of less tight and rigid fibrin network structures after maximal exercise, resulting in a possible decreased risk of a thrombotic event.

In the event of the meal intervention, changes in metabolic variables occurred that seemed to have an influence on the fibrin network structure. In this study, changes 1-hour post-meal indicated the formation of fibrin network structures that were shorter and thicker than the networks formed in plasma of sedentary males with the ingestion of a high GI meal. Fibrin network structures with improved compaction and permeability were formed in the active males with the low GI meal compared to those formed in sedentary males. When the maximal exercise was introduced the combination of the low GI meal in active males led to more permeable networks with shorter and thicker fibres. The tensile strengths were also lower. When the changes in recovery were determined, the active and sedentary males formed more permeable networks with the low GI meal.

6.3 Limitations of the study

One of the limitations in this study was that the sedentary males were not a control group for the active males. This was because of the significant differences in the BMI, body weight, and percentage body fat. This resulted in significant differences between the serum cholesterol and triglycerides concentrations of the active and sedentary males. The sedentary males had significantly higher total-cholesterol, LDL-cholesterol and triglyceride concentrations. The HDL-cholesterol of the active males was significantly higher than the concentrations of the sedentary males. When the effects of long-term physical activity is considered, it would be virtually impossible to find a control group for active subjects. This is due to the fact that the advantages of physical activity are a lower BMI, percentage body fat, total cholesterol, LDL-cholesterol, triglycerides and increased insulin sensitivity.

A further limitation was that a limited number of markers of the haemostatic balance were determined. A clearer interpretation of the results would have been possible had markers such as t-PA, PAI and fibrinogen degradation products for fibrinolysis been determined. Measurement of the fibrin content of the fibrin network structures would also have given more information of the fibrin network structure.

Finally, the small number of significant differences in the results obtained might be due to the age group and number of the subjects. It has been reported that in young, healthy adults changes in the haemostatic variables due to physical activity are limited, reasons being that the subjects are healthy with all the biochemical parameters within the normal range. Little changes can be expected in variables within normal concentrations.

6.4 Future research

This study is the first addressing the influence of physical activity on fibrin network structures during a diet intervention. This study indicated that physical activity results in different types of fibrin network structures forming *in vitro*. The ingestion of a pre-exercise meal with a different GI brought about changes in the circulation, which were

associated with changes in fibrin networks. This study has given an indication of the possible changes in fibrin network structures due to different pre-exercise meals together with changes due to physical activity. Possibilities for future research would include investigating the following aspects:

- studying a larger population
- studying an older population with possible "abnormal" networks and CHD risk factors
- changes that occur in the fibrin network structure with long-term physical training
- changes in the fibrin network structure with physical training and different diet interventions
- the effects of physical training on fibrin network structures in patients with early myocardial infarction which Fatah *et al.* (1996b) showed were resistant to fibrinolysis.

6.5 Recommendations and practical implications

In conclusion, the results of this study gave an indication that physical activity may favour the formation of less tight and rigid fibrin network structures that are less resistant to fibrinolysis. The ingestion of a low GI meal one-hour pre-exercise ensured a slow release of glucose with a delayed release of insulin. This, in combination with physical activity, might be beneficial for the person prone to the formation of detrimental blood clots in the circulatory system. It is thus suggested that regular physical activity seems to have a positive influence on the fibrin network structure. The changes that occurred in the fibrin network structure might be due to the changes occurring in response to glucose, insulin and triglyceride levels because of the physical activity. The benefits might be in the adaptation that occurs in the environment surrounding the fibrin, prior to the formation of the fibrin network structure.

The results from this study indicated that a maximal bout of exercise involves a risk for the formation of detrimental blood clots in the circulatory system. It seems important not to participate in a maximal activity when unfit, but to follow a training programme that gradually increases physical fitness. It also seems that the ingestion of a low GI meal has less detrimental effects than a high GI meal on fibrin network characteristics when

ingested pre-exercise by active males. Although the mechanism of the benefits of physical activity and diet has not been found, this study gave an indication that it might be through the fibrin network structure characteristics. More research is necessary for more conclusive answers.

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ADDENDUM A

INFORMED CONSENT FORM

INFORMED CONSENT

The school of Nutrition, Family Ecology and Physiology

1. **Project title**

Characteristics of the fibrin network structure of sedentary and active males

Project leaders

Sr. MC Lessing, registered nursing sister

Prof. HH Vorster: HOD Research

Prof. WJH Vermaak: HOD Chemical Pathology

Prof. CS Venter: HOD Nutrition

2. **The procedures that will be followed**

The subjects will have to fast for 10h to 12 h. Fasting venous blood samples for the baseline values will be drawn at three different periods. These periods will be before, during maximal activity and 30 minutes after activity was stopped. These procedures will be repeated with the consumption of a high glyceamic index meal before the maximal activity. The test will be repeated with the intervention of a low glyceamic index meal. During the pre-exercise meal intervention testing, four blood samples will be drawn. With these blood samples a total DAX profile will be done together with the determination of fibrinogen, Factor VII, insulin, and fibrin network structure characteristics. Mass, length, percentage body fat, blood pressure and heart rate will be measured.

3. **Inconvenience and danger that are involved in the project**

Blood has to be drawn as described in paragraph 2. All the necessary precautions will be taken to ensure that the process is performed with the least amount of inconvenience.

3. **Precautions taken to protect the subjects**

A qualified en experienced nurse will draw the samples according to a set protocol to prevent the contamination of any infectious diseases.

To the undersigned of the informed, consent:

1. You are invited to participate in the research project as described in paragraph 2 in the previous section. It is important that you will read and understand the following principles of the research project that are applicable to all participants:

1.1 Participation in the project is completely voluntarily

1.2 It is possible that you will not gain any personal advantage from the project, although the knowledge that is gained from the project may be to the advantage of other people.

1.3 You are free to withdraw from the project without any reason. You are kindly requested not to do so without giving it good thought, as the statistical validity of the project will be influenced.

1.4 A summary of the type of project, risk factors and possible inconvenience that may occur together with the advantages are given in part 1

1.5 You are welcome to ask any questions at any stage, concerning the project and the procedures, to the project leaders. The project will also be fully discussed with you.

CONSENT

I, the undersigned_____have read the preceding information regarding the project as described in part I paragraph 2 together with an oral explanation and declare that I understand it. I was given the opportunity to discuss in short the aspects of the project with the leader. I herewith declare that I voluntarily participate in the research project.

_____Signed at_____

(Signature of volunteer) on _____

_____Signed at_____

(Signature of project leader) on _____

_____Signed at_____

(Signature of witness) on _____

ADDENDUM B

RISK SCREENING FORM

RISK SCREENING

1. Are you capable of walking 2 – 3km without experiencing chest pains and/or becoming short of breath?

Yes **No** **Uncertain**

2. If No, state the problem

_____

3. Has your doctor indicated that you have a heart ailment?

Yes **No**

4. If yes, state the problem

_____

5. Do you suffer from high blood pressure?

Yes **No**

6. Are you taking medication on a regular basis?

Yes **No**

7. If yes, what medication and for what reason?

_____

8. Are you experiencing any pain in the lower back area?

Yes **No**

9. If yes, please specify

_____

10. Are you troubled by any kind of hernia?

Yes **No**

11. If Yes, please specify

_____

12. Do you suffer from any of the following ailments?	**Yes**	**No**
a) Exercise induced asthma	☐	☐
b) Regular headaches or migraine attacks	☐	☐
c) Regular tiredness	☐	☐
d) Depression	☐	☐
e) Kidney problems	☐	☐
f) Cramps in the legs	☐	☐
g) Diabetes Mellitus	☐	☐
h) Flu, cold or bronchitis	☐	☐
i) Joint or skeletal problems	☐	☐
j) Epilepsy	☐	☐
k) Varicose veins in the legs	☐	☐
l) Any pulmonary ailments	☐	☐
m) Dizziness or fainting	☐	☐
n) Blood circulatory disorder	☐	☐

13. Please mark the one condition most applicable to you

Never ever tense	
Seldom tense	
From time to time tense	
Frequently tense and/or anxious	
Usually tense and/or anxious	
Exceedingly tense and anxious/ Take medication	

14 Has your doctor diagnosed any other medical problem or ailment, which seems to be necessary to be considered or might affect your exercise programme?

Yes **No**

15 If yes, please specify

_____

16 Are you a smoker?

Yes **No**

17 If yes, how often per day?

_____

18 If no, have you ever smoked and when did you stop?

Yes **No**

19 Do you participate in sport?

Yes **No**

20 If yes, please specify sport and intensity.

_____

21 Do any of your blood relatives suffer from any of the following conditions?

CONDITION	FATHER	MOTHER	BROTHERS	SISTERS
High cholesterol levels				
Increased triglycerides				
Coronary heart disease under 60				
Diabetes Mellitus				
Hypertension				
Overweight				
Stroke				

ADDENDUM C

DATA SHEET

DATA SHEET

No.:_____

NAME:_____SURNAME:_____

ADDRESS:_____TEL: (h)_____

_____ (w)_____

_____ (cell)_____

D.O.B:_____AGE:_____

ACTIVITY:_____TRAINING SESSIONS _____ days/week

DISTANCE_____km/week

STATURE:_____MASS:_____

RESTING HEART RATE:_____RESTING BLOOD PRESSURE_____

AGE ADAPTED MAX.HEART RATE:_____bpm

SKINFOLDS

Triceps			
Subscapula			
Supra- spinale			
Para-umbilicus			
Mid thigh			
Medial calf			

PHYSICAL ACTIVITY

RESISTANCE	HEART RATE	BLOOD PRESSURE	BORG SCALE
Rest			

ADDENDUM D

DIETARY INTAKE QUESTIONNAIRE
(24HR-RECALL)

DIETARY INTAKE QUESTIONNAIRE (24 HR-RECALL)

1. Name of subject:_____ ☐☐☐☐ 1 2 3 4

2. Date of birth: Day_____Month _____Year _____ ☐☐☐☐☐☐ 5 6 7 8 9 10

3. Day of the week interviewed:

MON ☐ 1 TUES ☐ 2 WED ☐ 3 THUR ☐ 4 FRI ☐ 5 SAT ☐ 6 SUN ☐ 7 ☐ 11

4. Was yesterday typical/routine for you? Yes ☐ 1 No ☐ 2 ☐ 12

If not, why?_____ ☐ 13

5. What kind of margarine do you usually eat:

- on bread: B-6502 ☐ HM-6508 ☐ MED-6560 ☐ PM-6521 ☐ Ghee-6554 ☐ ☐☐☐☐ 14 15 16 17
- in cooking: B-6502 ☐ HM-6508 ☐ MED-6560 ☐ PM-6521 ☐ WF-6545 ☐ ☐☐☐☐ 18 19 20 21

Ghee-6554 ☐

6. What kind of bread do you usually eat?

White ☐ 4001 Brown ☐ 4002 Whole wheat ☐ 4003 ☐☐☐☐ 22 23 24 25

7. What kind of milk do you usually drink?

SM-0007 ☐ WM-0006 ☐ BL-0068 ☐ Breast-0029 ☐ ☐☐☐☐ 26 27 28 29

Cond-WM-0002 ☐ Cond-SM-0032 ☐ Goat-0026 ☐ 2%-0069 ☐ Formula:_____

Other (specify)_____

8. Did you eat at a feeding scheme or residence yesterday? Yes ☐ 1 No ☐ 2 ☐ 30

(If yes, fill in the last page)

9. Name of Interviewer:_____ ☐☐ 31 32

Now I want you to tell me everything you ate and drank yesterday. Let us start with when you woke up. Did you have anything to eat or drink? Proceed through the day following the subject's activities. When you have finished, summarise it for the caretaker. Any forgotten items can then be added.

Instructions: Enter each item eaten in grams under the correct interval of the day eaten. Make sure that the code is entered. Items not on the questionnaire should be looked up in the FOOD COMPOSITION TABLES. Specify fully when new items are entered and look up the code later. Recipes should be added on page 13.

This questionnaire was updated from the one developed by N.P. Steyn (Steyn NP. Dietary intake and nutritional status of 11-year-old children in the Western Cape. Stellenbosch University: Ph D. thesis; 1988).

ABBREVIATIONS

Measures
1t = 1 rounded teaspoon
1T = 1 rounded tablespoon (15ml)
1 LS = 1 rounded servingspoon (30ml)
c = measuring cup (250ml)
s/s =small size
m/s = medium size
l/s = large size
E = enriched
P = plain

Milk:
SM = skim milk
WM = whole milk
BL = blend
Con = condensed

Bread:
Wh = white
Br = brown
Ww = wholewheat

Meat:
F = with fat
FT = fat trimmed

Oil/Fat:
B = butter
HM = hard margarine
Med = medium fat/light
PM = polyunsaturated
VO = vegetable oil
WF = white fat

BR = Breakfast (Up to 09h00)
IS = Inbetween snack
L = Lunch (midday (12h00 – 14h00)
D = Dinner (evening) (17h00 – 19h00)
AD = After dinner
Comm = commercial
Home = home made
Pot = potato
Cab = cabbage
Carr = carrot
Fill = filling
Usually = At least 4×/week

	FOOD ITEMS	CODE	QUANTITY (g/ml)	BR	IS	L	IS	D	AD
TEA & COFFEE	Tea:9514; Rooibos 9560; Coffee9513		Teacup=180ml;Mug=250ml						
	+ Sugar	9012	1t = 6g						
	+Condensed milk: SM-0032; WM-0002		1t = 10g						
	+ Non-dairy creamer	0039	1t = 3g						
	+ Milk blend	0068	20ml – tea in cup 35ml – tea in mug 40ml – coffee in cup 75ml – coffee in mug						
	+ SM-0007; WM-0006; 2%-0069; Breast-0029; Goat-0026								
	+ Formula milk (Specify)								

MILK & MILK DRINKS	Buttermilk	0001	S/s = 175ml L/s = 500ml ½c = 125g						
	Maas/Amazi/Sourmilk	0085							
	Custard:SM-0005;WM-0004								
	Milk:SM-0007;WM-0006;Bl-0068;2%-0069;Breast-0029;Goat-0026;Formula (Specify) No. of scoops/bottle used:		To drink ½c = 125ml Baby bottle = 250ml						
	+ sugar	9012	1t = 6g						
	+ ice cream-6548;Sorbert-6516		1 Scoop = 40g						
	+ Sustagen-9722;Complan-9725		2 Scoops = 25g;1T = 15g						
	+ Milo/Cocoa/Horlicks/Ovaltine	0024	1t = 5g						
	Yoghurt:plain SM-0022;WM-0045		S/s = 175ml Yogi sip = 350ml ½c = 125g						
	Flav-0044; Fruit-0020								

COLD DRINKS/ JUICE	Apple Juice – No sugar	7080	Liquifruit s/s = 250ml L/s = 500ml Ceres s/s = 200ml Carton/bottles s/s = 350ml L/s = 500ml						
	Apricot:+sugar-7008;No sugar-7040								
	Mango-7162;Granadilla-7159;Grape-7169								
	Orange:+sugar-7033; No sugar-7113								
	Guava: + sugar-7024; No sugar-7103								
	Peach-7117;Pear-7120;Naartjie-7161								
	Cold drinks: Squash-9002;Magou-9562; Carbonated-9001		S/s bottle = 300ml L/s bottle = 500ml S/s can = 340ml						
	Low-cal-9013; Syrup(undiluted)-0534								

BREAKFAST CEREALS	Mabella; Soft or stiff	4034	½c = 125ml						
	M/Meal: Soft: plain-4254; Enriched-4330		1c soft = 250g						
	Stiff: Plain-4255; Enriched-4331		1c stiff = 250g						
	Crumbly :plain-4256; Enriched-4332		1c crumbly = 140g						
	Oats-4032; Tastee wheat-4033; Sour porridge		½c = 125g						
	Corn Flakes-4036;Sugar Frosted-4218		1c = 40g						
	Honey crunch and muesli	4122	½c = 65g						
	Pronutro: Great Start-4316; High energy-4038; Wholewheat-4314		½c = 50g						
	Puffed Wheat-4149; Sweetened-4221 (Honey smacks)		½c = 12g						
	Raisin Bran-4217; Fruit loops-4303		Raisin Bran ½c = 45g						
			Fruit Loops ½c = 18g						

	FOOD ITEMS	CODE	QUANTITY (g/ml)	BR	IS	L	IS	D	AD
BRE AKF AST CER EAL S	Special K-4146; All Bran-4035		½c = 25g						
	Rice Crispies-4046; Cocopops-4216		½c = 20g						
	Weetbix	4037	1 = 25g						
	+ Fat: B-6502: HM-6521; WF-6545; Med-6560								
	Milk: SM-0007; WM-0006; Bl-0068;2%-0069; Goat-0026; Breast-0029; Formula – Specify:		With instant = 125ml With porridge = 60ml With pronutro = 180ml						
	+ Sugar	9012	1t = 6g						

BRE AD & RO LLS	Bread: Comm & Home: Wh-4001; Br-4002; Ww-4003		Wh + Br 10mm = 30g Ww 10mm = 35g Wh + Br 20mm = 60g Ww = 20mm = 70g						
	Cream crackers-4022; Provita-4027		1 = 7g						
	Maize meal bread	4083	M/s = 30g; L/s = 50g						
	Rolls: Wh-4001; Br-4002; Ww-4003 Roti: VO-4199; HM-4198		Wh round (10cm) = 30g Wh long (16cm) = 40g S/s = 50g (Roti)						
	Rusks: Comm Plain	4206	1 = 15g						
	Comm Buttermilk: Wh-4160; Ww-4161		Wh = 35g; Ww = 30g						
	Home ButtermilkL Wh-4006; Ww-4049		Wh = 30g; Ww = 30g						
	Scones: (Wh) SM-4269; WM-4029		6cm diam = 35g						
	(Ww) SM-4270; WM-4142		8cm diam = 60g						
	Vetkoek: Wh-4057; Ww-4148		8cm diam = 60g						

SPR EA DS ON BRE AD			Thin Med Thick						
	Beef Fat-6519; Mutton Fat-6522; Butter-6502; Ghee-6554; Lard-6520; WF-6545		5 10 15						
	Fishpaste-2567; Liver spread-1517		5 7 10						
	Jam-9008; Honey-9007; Syrup-9011		10 20 35						
	Marg: H-6508; Med-6560; PM-6521		5 7 10						
	Marmite-9502; Meat spread-9501		2 4 7						
	Peanut butter-6509; Sandwich spread-6551		5 10 20						

EG GS	Eggs : Boiled	1001	1 egg = 50g						
	Curried	1037	1 egg + sauce (1 T) = 75g						
	Fried: B-1002; HM-1012; PM-1013		1 egg = 52g						
	VO-1003; Bacon Fat-1004								
	Scrambled/Omlette: SM+B-1021; SM+HM-1022; SM+PM-1023; SM=+VO-1024; WM+B-1009 WM=HM-1025; WM+PM-1026; WM+VO-1008		IT = 35g; 1LS = 80g ½c = 115g (± 2 eggs) Omelette= 60g egg med 120g (L/S)						

	FOOD ITEMS	CODE	QUANTITY (g/ml)	BR	IS	L	IS	D	AD
CHEESE	Cheddar-0010; Sweetmilk-0011		Grated: med = 10g Thick = 15g 1 cheezi = 20g; cubes = 30g 1 slice = 8g						
	Cheese spread	0018	Med = 12g; thick = 25g						
	Cottage cheese: creamed	0047	Thin = 10g; med = 20g						
	Fat free-0017; Low fat-0048 (smooth) (chunky)		Med = 20g; thick = 30g						
	Macaroni cheese: SM-4176; WM-4120		1T = 45g; 1LS = 90g ½c = 115g						
	Pizza (Cheese + Tomato)	4193	S/s = 90g; L/s = 340g						
	Savoury Tart: + Asparagus-4210; + Vienna-4153		Wedge small = 65g Med = 75g Large = 110g						
	+ Tuna-4209								
MEAT	Bacon: Fried: Lean-1510 F-1501		1 rasher = 10g						
	Beef: Corned/Silverside/Cold cuts;F-1519 Lean-1558; Curry Beef-combine codes		138 × 85 × 3 = 20g ½c = 100g						
	Fillet: F-1528; FT-1524		100 × 70 × 10 = 90g						
	Mince: Pan Fried F-1505; Lean-1556; Curry-1631		T = 40g; LS = 85g ½c = 100g						
	- Savoury (Tomato + Onion)	1585							
	- Cottage Pie: WM + HM	1623							
	Roast: F-1539; FT-1555		120 × 60 × 5 = 35g 120 × 60 × 10 = 70g						
	Rump: Fried:F-1503; FT-1554		S/S 130 × 70 × 15 = 125g LS 165 × 70 × 30 = 270g						
	Sirlion/T-Bone:Grilled:FT-1541;FT-1502								
	Stew: Cabbage + Onion + Potato	1619	1 LS = 105g; ½c = 125g						
	: Pot + Carrots + Peas + Onions	1504							
	Biltong: Beef-1506; Game-1507		Grated 1LS = 10g Beefeater = 18g Sliced 1 LS = 35g						
	Bobotie:Lean,SM,VO-1628;F,WM,VO-1584		1 LS = 85g; ½c = 115g						
	Chicken: Boiled + Skin-1621; No skin-1560; Curry-combine		Breast + skin = 125g Thigh = 80g Drumstick = 42g Foot = 30g Wing = 30g Pie(comm)=150g;home = 90g Liver = 30g; stomach = 20g						
	Feet-1609; Giblets-1610; Heads-1611								
	Pie (comm)	1549							
	Roast + Skin-1520; No skin-1545								
	Stew:Carrot, peas, pot-1618 Cabbage + Potato-1619 Tomato + Onion-1583		1 LS = 90g; ½c = 125g						
	Batter dipped-fried eg Kentucky	1634	1 LS = 105g; ½c = 125g						
	Cornish Pie: (comm)	1548	Med = 150g						
	Frankfurter	1532	155 × 20 = 45h 168 × 21 = 60g						
	Goat meat: Stewed (plain)-1677 + Veg-1678		120 × 60 × 5 = 35g 120 × 60 × 10 = 70g						
	Ham-1564; Ham & Tongue loaf-1600		Med slice = 25g						
	Heart: Beef-1565; Sheep-1566 Kidney: Beef-1518; Sheep-1551 Lung: Beef-1635		Sheep heart = 60g Sheep kidney = 30g Beef kidney = 85g						
	Lasagne: SM-4315; WM-4061		T = 40g;LS = 75g; ½c = 120g						

	FOOD ITEMS	CODE	QUANTITY (g/ml)	BR	IS	L	IS	D	AD
ME AT	Liver: Fried: Beef-1515; Sheep-1550		Sheep = 55g Chicken = 30g Beef = 80g						
	Cooked:Chicken-1567;Patty-1568(fried)								
	Meat ball;F+Egg-1562;F-No egg-1563; Lean-1653		50mm = 60g; 75mm = 120g						
	Meat loaf: F-1655; Lean-1615		80 × 85 × 15mm slice = 80g						
	Meat patty: Hamburger	1581	S/s = 50g; m/s = 100g						
	Mutton: Chop (grilled) F-1522;FT-1529		Loin chop = 60f Rib chop = 40g						
	Roast: F-1542; FT-1570		S/s slice = 30g Med = 70g						
	Stew:Plain-1571;Irish-1511(Carrots+Pot) Curry-1660; Greenbean-1661		1 LS = 105g; ½c = 125g						
	Offal: Cooked-Tripe; Tongue, Harslag, Pens,Vetderm (Specify):	1616	1 LS = 105g; ½c = 125g						
	Oxtail: Stewed	1573							
	Polony	1514	Slice 5mm thick = 8g Comm slice = 16g						
	Pork: Chop(Grilled)F-1525; FT-1574		Chop: 115 × 80 × 20 = 100g Schnitzel: 115×80×20 = 110g Roast: 110 × 65 × 5 = 30g 1 LS = 105g; ½c = 125g 3 Ribs = 130g						
	Crumbed-1602; Spareribs-1625								
	Roast: F-1553; FT-1575								
	Salami and Russians	1543	Slice 5mm thick = 12g 1 Russian = 50g						
	Samoosa: with Veg-4272; Meat-4196		S/s = 42g						
	Sausage: Beef:Dry-1544; Fried-1526 (Boerewors)		Thin × 200mm = 45g Thick × 165mm = 90g						
	Pork: Fried/Grilled	1527	Med = 55g						
	Roll/Meat Pie (Comm)	1534	25mm Pie = 120g Roll × 135mm = 165g						
	Spaghetti Bolognaise: Lean-4236; F-4060		T= 40g; LS = 75g; ½c = 100g						
	Steak & Kidney: Pie-1552; Stew-1576		Comm pie = 120g (30mm) 1 LS = 100g; ½c = 135g						
	Tongue: Ox-1530; Sheep-1577		Slice 75 × 45 × 10 = 40g						
	Toppers/Imana: Cooked	3527	LS = 85g ; ½c = 120g						
	Veal: Cutlet (Fried); Plain-1671; Crumbed-1580		1 Chop = 90g						
	Vienna sausage/ Canned sausage	1531	100mm = 30g; 150mm = 40g						
	Worms/Insects: Mopani-1676; Specify								
	Wild birds, Animals; Specify								
	Other								

FIS H	Bokkems	2551	1 s/s = 25g (120mm) L/S = 40g (135mm)						
	Fatty fish: Kipper;Galjoen; Snoek; Shad		Small 50 × 55 × 30 = 60g Med 100× 55 × 30 = 120g Stew 1 LS = 95g; ½c = 140g						
	:Fried VO-2535; Batter-2548; Grill-2533; :Smoked-2570 :Salted-2551; Steam-2559; :Stew-2527 (Tomato and Onion)								
	Fish cake:Fried:Home-2552;Comm-2531		65 × 15mm = 50g						
	Fish fingers: Fried	2531	85mm = 35g						
	Haddock: Smoked (Boiled)	2511	70 × 70 × 15 = 65g						
	Pilchards:Tomato sauce-2557;Brine-2503		1 = 75g						

	FOOD ITEMS	CODE	QUANTITY (g/ml)	BR	IS	L	IS	D	AD
FIS H	Sardines: + Sauce-2539; + Oil-2560		S/s = 7g; L/S = 25g						
	Smoorsnoek:	2525	1 L/s = 55g; ½c = 80g						
	Sole: Fried-2542; Grilled-2524		Baby sole: 180mm = 70g						
	Tuna: Oil Pack-2547; Water-2501		¼c = 50g						
	White Fish:Hake,haddock, Kingklip, Cod		S/s piece 50 × 55 × 30 = 60g Med 100 × 55 × 30 = 120g Stew 1 LS = 95g; ½c = 140g						
	Stew-2527 (Tom+On); Baked-2545								
	Grilled-2530; Batter-2523; Fried-2509								
	Other: e.g. Fresh water fish; Specify								

			T	LS	½c						
STA RC H	Maize :Stiff(E)-4331;Crumbly (E) 4332 Stiff(P)-4255;Crumbly(P)-4256		Stiff	75	120	125					
	Mabella/Sorghum:	4315	Crum	30	75	70					
	Maize rice:	4043		25	45	65					
	Samp: (Cooked)	4043		55	125	125					
	Rice: Wh-4040; Br-4134			25	60	65					
	Spaghetti/Macaroni: (Cooked)	4062		35	70	90					
	Spaghetti + Tomato sauce	4058		45	80	125					
	Stamped wheat/Wheat Rice	4042		30	80	80					
	+Fat: B-6502; HM-6508;PM-6521; WF-6545; VO6510;Med-6560										
	Ghee-6554										

LEG UM ES AN D SO UP	Baked beans	3504		50	150	135					
	Beans: (Cooked) Haricot-3515; Sugar-3542; Kidney-3513			50	85	135					
	Breyani: Rice+Lentils+Ghee-3524; +VO-3523			40	80	85					
	Lentils: Cooked/curried-3509										
	Samp and beans (1:1)-4257										
	Soup: Comm-3054					125					
	Split pea-3045; Lentil-3041; Beef+Veg-3047; Bean-3033			35	80	130					
	"Sousboontjies"	3502		40	105	135					
	Stew: Bean + Potato + Onion	3508		60	120	125					

		Boiled	Fat Added (or Fried)												
CO OK ED VE GET AB LES		NF	B	HM	PM	VO		T	LS	½c					
	Gr Beans	8002	8080	8095	8099	8100		25	60	80					
	Gr Beans Curry	8101					8101	40	75	120					
	Gr Bean + Potato + Onion		8003	8102	8103	8104									
	Beetroot+sugar	8005					8005	40	70	80					
	- No sugar	8004					8004								
	Brinjal	8006	8109	8110	8111	8112		1 slice = 20g (70mm) + batter = 30g							
	-Fried + Egg						8113								
	-+Tomato+Onion		8105	8105	8107	8108		50	100	130					
	Broccoli	8007	8114	8115	8116			25	60	75					

	FOOD ITEMS						CODE	QUANTITY (g/ml)			BR	IS	L	IS	D	AD
		NF	B	HM	PM	VO		T	LS	½c						
COOKED VEGETABLES	Brussels Sprouts	8009	8117	8118	8119			50								
	Cabbage	8006	8012	8120	8121	8122		30	55	80						
	Cab+Pot+Onion		8014	8123	8124	8125		35	75	80						
	Carrots	8067	8074	8126	8127	8082		20	50	80						
	Car+Pot+Onion		8073	8132	8133	8134		35	70	105						
	Carrot+sugar	8067	8019	8129	8130	8131		25	50	85						
	Cauliflower	8203	8021	8135	8136	8137		40	65	80						
	Caul+Cheese	8022					8022	43	70	90						
	Marog/imifino*	8302					8302	40	105	90						
	Mealies(corn)	8033					8033	30	60	95						
	"Sweetcorn"	8034					8034	55	125	135						
	Mix veg Tin/Froz	8035	8144	8145	8146	8147		35	75	75						
	Mushroom sliced	8037	8148	8149	8150	8151		30	65	80						
	Onions sliced	8083	8153	8154	8155	8038		50								
	Onion+ Batter						8156	Rings: med = 40g								
	Peas	8026	8075	8166	8167	8168		30	65	85						
	Peas + Sugar	8027	8032	8169	8170	8171		30	65	85						
	Potato + Skin	8046					8046	S/s = 60g; m/s = 90g								
	Baked + Skin	8044					8044	S/s = 60g; m/s = 90g								
	Chips					8048	8048	½c = 50g; med = 80g								
	Peeled	8045	8076	8177	8178	8179		S/s = 60g; m/s = 90g; (90 × 60 × 40)								
	Sauté		8180	8181	8182	8183		3	50	90						
	Potato cake					8234	8234	1 med = 40g (75 × 30)								
	Pot mash (SM)			8185	8186			50	115	125						
	Pot mash (WM)		8047	8187	8188											
	Pot (Roast): Beef fat-8189; Chicken-8242; Lamb-8043; Pork-8275							1 med = 70g								
	Pumpkin &Butternut	8069	8050	8202	8203	8081		45	85	105						
	Pump + Sugar	8036	8051	8204	8205	8206										
	Pump fritter					8094	8094	75 × 50 × 9 = 25g								
	Spinach	8071	8055	8209	8210	8211		40	105	90						
	Spin+Onion+Potat		8056	8212	8213	8096		50	105	110						
	Squash-Gem/Marrow	8070	8052	8193	8194	8195		½ gem = 45g								
	Squash+Sugar	8063	8053	8196	8197	8198		1 LS marrow = 85g								
	Sw potato	8214					8214	50	110	145						
	Sw Pot + Sugar	8243	8072	8058	8215	8215										
	Tomato+Onion	8244					8244	35	75	140						
	Tom+Onio+Sugar	8221					8221									
	Tomato		8218	8219	8220	8077		1 slice 5mm=15g(thin) Med = 25g								
	Turnips	8222					8222	25	45	90						
	Other															

	FOOD ITEMS	CODE	QUANTITY (g/ml)	BR	IS	L	IS	D	AD
SALADS	Asparagus-8001; Avocado-7132		¼ avo(80×50mm)=40g med asparagus = 15g						
	Beetroot (Grated) + Sugar	8005	1T = 25g; Ls = 65g						
	Carrots: Raw-8015, + Sugar-8028 (Grated)		1T = 25g						
	+ Pine + Orange-8016; + Apple + Orange-8017		1T = 35g; 1LS = 60g						
	Coleslaw + Mayonnaise	8011	T = 20g; LS = 40g; ½c = 50g						
	Cucumber Raw/Pickled	8025	Med slice=10g; thick=15g						
	Lettuce	8031	1 med leaf = 30g						
	Mixed (Tom + Cucum + Lett) – No dressing	8240	1 T = 40g						
	Mixed Green – no dressing	8246	1 LS = 85g						
	Potato salad + Mayonnaise (comm)	8247	T = 45g; 1LS = 105g ½c = 120g						
	Tomato (Raw)	8059	Med=120g; slice =15g						
	Other								

DRESSINGS	French dressing	6512	1t = 5g; 1T = 15g						
	Mayonnaise; Home-6536; Comm-6513		1t = 10g						
	Mayonnaise: low fat	6514	1T = 40g						

	FRUIT	Canned+ Sugar	Raw	Dry	Stewed								
FRUIT	Apple	7073	7001	7074	7077		1T = 60g; ½c = 120g						
	Apricot	7004	7003	7005	7006		1 med = 35g						
	Banana		7009			7009	1 med = 75g						
	Dates		7012			7012	1 med = 10g						
	Figs		7013	7027			1 med = 40g (45× 44) 1 dry = 20g						
	Fruit salad	7051	7079	7066	7062		½c = 110g (med)						
	Granadilla		7014			7014	1 med = 22g						
	Grape fruit	7017	7016				½ med = 125g						
	Grapes		7020			7020	Med bunch=230g; ½c=90g						
	Guava	7023	7021				Med (6cm) = 95g						
	Litchi		7107			7107	Med (3cm) = 8g						
	Mango		7026			7026	135mm = 350g						
	Naartjie	7110	7028				Med = (5cm) = 75g						
	Orange		7031				Med (7cm) = 180g						
	Paw-paw	7114	7034				Wedge 165×26×27=90g						
	Peach	7038	7036	7039	7040		1 med = 150g(60×65)						
	Pear	7054	7053	7056	7057		1 med(8065mm)=165g						
	Pineapple	7123	7052				1slice(85×10mm)=40g						
	Plum		7041			7041	1 med = 50g (45×40)						
	Prunes	7154		7069	7035		1T = 50g; ½c=110g; 1=12g						
	Raisins		7022				Handful = 27g						
	Strawberries	7129	7044				1 med =12g; ½c = 80g						
	Sweetmelon		7046				1wedge145×31×20mm = 60g; ¼c = 110g						
	Watermelon		7047			7014	Slice330×70mm=220g						
	Wild Fruit, Berries Specify:												

	FOOD ITEMS			CODE	QUANTITY (g/ml)	BR	IS	L	IS	D	AD
		SM	WM								
PUDDINGS	Apple + Batter	4178	4154		Med serving = 70g						
	Apple crumble		4165		Med serving = 70g						
	Baked Pudd + Syrup	4181	4131		Med serving = 30g						
	- no syrup	4180	4013		30 × 65 × 65 = 50g						
	Blancmange	4090	4089		LS = 75g; ½c = 95g						
	Egg type e.g. Bread sago	4179	4063		1T = 50g; ½c = 140g LS = 100g						
	Ice cream			6507	Scoop = 40g;1LS=65g ½c = 75g						
	Instant pudding	4133	4066		1 T = 45g; LS = 95g; ½c = 145g						
	Jelly			9004	1 T = 35g; 1LS = 75g ½c = 110g						
	Jelly + Fruit			9033	1T = 40g; 1LS = 90g ½c = 125g						
	Jelly Whip	0037	0038		1T = 55g; 1 LS = 95g ½c = 120g						
	Pancakes/ Crumpets	4177	4030		1 crumpet = 25g pancake = 70g						
	Soft serve			6547	Plain = 135g; + flakes = 155g						
	Sorbet			6516	1LS = 65g; ½c = 75g						
	Trifle-4130; Vermicelli Pudding-4233				½c = 130g (med)						

SAUCES	Cream: Plant-6517; Canned-6524 Light-6504; Heavy-6503		1 T = 30g							
	Chocolate sauce	3016	1 T = 15g							
	Custard: SM-0005; WM-0004		1 T = 13g; LS = 40g							
	Sugar	9012	1t = 6g							
	Other									

CAKE	Banana Loaf: WM + HM-4164; SM + PM-4214		Slice = 45g; 90×80×10mm							
	Cake - carrot	4244	80 × 40 × 40 = 50g							
	Plain: SM + B-4009; HM-4095; PM-4096		Single slice = 50g (75 × 75 × 20mm) Double slice = 100g Icing = 10g per slice							
	WM + B-4009; HM-4097; PM-4101									
	Cake icing: HM-9042; PM-9043									
	Chocolate (No icing) WM-4099; SM-4170									
	Fruit: Comm-4102; Home-4305		Home: =70g (70 × 85 × 15mm) Comm: =35g (90 × 70 × 15mm)							
	Sponge (Plain)	4011	100 × 50 × 50 = 40g							
	Swiss roll	4103	Slice= 60g;15mmthick							
	Cheese cake; Baked-4108; Unbaked-4109		Slice = 70g (95 × 50 × 30mm							

COOKIES	Comm + fill-4008; Plain-4007		Plain = 10g +fill = 15g							
	Home: Plain HM-4025; PM-4172		Plain = 15g +fill = 20g Hertzog =50g; Cupcake = 35g Shortbread = 12g							
	Jam-4110; Oats-4065; Shortbread-4111									
	Custard slice	4169	110×45×35mm = 250g							
	Date loaf; HM-4054; PM-4171		Slice =40g 90×75×10mm							
	Doughnuts: Jam-4301; Plain-4024		Med round = 45g Med long = 90g							
	Eclairs + cream + chocolate	4070	1 = 120g (160mm)							

	FOOD ITEMS	CODE	QUANTITY (g/ml)	BR	IS	L	IS	D	AD
COOKIES	Gingerbread: HM-4047; PM-4215		90 x 75 x 15 =70g						
	Koeksister	4023	100 x 35 = 60g						
	Raisin bread	4005	Slice = 30g 85 x 100 x 10mm						
TARTS	Apple: HM-4016; PM-4192		50 x 50 x 50mm = 70g						
	Coconut	4020	50 x 100x30mm = 55g						
	Condensed: HM-4109; PM-4317		95 x 70 x 30mm 90g						
	Fridge (Fruit): HM-4246; PM-4312								
	Lemon meringue: HM-4018; PM-4184		100 x 70 x 35mm=75g						
	Milk (Short) WM + HM-4202; SM + PM-4189		120 x 70 x 25mm=75g						
	Milk (Flaky) WM + B-4321; WM + HM-4021								
	Savoury: Aspar-4210; Tuna-4209; Vienna-4153		120 x 50 x 25 = 75g						
	Tipsy: HM-4147; Jam-4017		87 x 70 x 50mm = 90g						
SWEETS	Bubble/Chewing gum	9019	See manual						
	Chocolates: Assorted	9017							
	Coated Bars eg Tex, Lunch, Chomp	9024							
	Milk eg. Smarties, Flake, Aero	9010							
	Nuts/Raisins	9020							
	Plain	9030							
	Dry fruit sweets	9021							
	Fruit gums	9027							
	Hard/Jelly sweets eg. Sugus, jelly tots, Fruit drops	9009							
	Ice Lollies	9002							
	Marshmallows	9028							
	Meringues	9035							
	Peanuts-6007; Peanut Brittle-9029								
	Peppermints	9031							
	Popcorn; Plain-4163; Sugar coated-4201								
	Potato crisps eg. Simba, O'Gradys	4275							
	Snacks – Fritos, Nicknaks	4067							
	Soft sweets – Fudge, Toffees, Caramel	9014							
OTHER	Cheese sauce: WM + HM-3012; SM + PM-3015		LS = 65g; 1T = 25g						
	Curry sauce	3017	1T = 25g						
	Chutney-3057; Atjar-3004	3057	1T = 14g; 1T = 60g						
	Gravy: Comm-3006; Meat-3009; NF-3008		1T = 15g; LS = 35g						
	Mustard	9509	1t = 6g						
	Tomato sauce (comm)	3027	1t = 6g						
	White sauce: WH + HM-3030; SM + PM -3029								

	FOOD ITEMS	CODE	QUANTITY (g/ml)	BR	IS	L	IS	D	AS
INF ANT FOO DS	Baby cereals (dry): Nestum 1-0501; 2-0503 Purity mixed-0511; Wholewheat-0503; Rice-0531 Cerelac-0505; Nestum Rice & Maize-0504 Junior-0502		1t = 2g 1T = 8g ½c = 20g						
	Milk: SM-0007, WM-0006; BI-0068; 2%-0069; Breast-0029; Goat-0026, Formula (Specify): No. of scoops/bottle used:		To drink ½c = 125ml Baby bottle = 250ml						
	+ sugar	9012	1t = 6g						
	First food fruit-0521; First Food Veg-0520		Jar = 80g; 1t = 1g						
	Fruit Juice (Strained)-0529; Fruit Juice-0535		½c = 125ml						
	Infant dinners(Dry):Beef + Veg–0510; Chicken + Veg-0509 Guava + Custard-0506; Mix veg-0508; Orange + Banana-0507		1t = 5g 1T = 15g ½c = 47g						
	Junior Food (Jar): Veg + Meat-0517; Mix Veg-0518; Pasta + Beef-0519 Junior Fruit (Jar): Fruit-0532; Guava-0524 Junior pudding: Fruit + Yog-0527; Vanilla Cust-0528		Jar = 200g 1t = 11g ½c = 125g						
	Strained Food (Jar): Macaroni Beef-0514; Veg + Meat-0515 Fruit + Yog-0526; Fruit-0523 Av. Pudding-0513; Meat soup-0516; Veg soup-0512; Vegetables-0522; Junior Fruit Guava-0525		Jar = 125g 1t = 11g ½c = 125g						

OTH ER									

ADDENDUM E

BASELINE VARIABLES OF THE BLOOD BIOCHEMISTRY OF THE ACTIVE AND SEDENTARY MALES

Table 1: The baseline variables of the blood biochemical analyses for the active and sedentary males

Variables	Active (n= 15)	Sedentary (n = 14)	Normal range$^{\phi}$
	Mean ± SD	Mean ± SD	
Sodium (mmol/L)	141.97 ± 1.31	141.90 ± 0.88	137 – 144
Potassium (mmol/L)	3.97 ± 0.21	4.04 ± 0.25	3.6 – 4.7
Chloride (mmol/L)	104.48 ± 1.92	105.07 ± 1.42	98 – 108
Total carbon dioxide (mmol/L)	23.66 ± 1.83	23.00 ± 1.96	23 – 29
Anion gap (mmol/L)	13.94 ± 1.23	13.91 ± 1.76	7 – 14
Ureum (mmol/L)	6.79 ± 1.21	5.16 ± 1.06	3.1 – 7.8
Creatine (μmol/L)	114.99 ± 12.78	112.45 ± 9.44	81 – 114
Uric acid (mmol/L)	0.38 ± 0.09	0.35 ± 0.11	0.31 – 0.47
Total calcium (mmol/L)	2.32 ± 0.06	2.37 ± 0.10	2.20 – 2.55
Correlated calcium (mmol/L)	2.28 ± 0.04	2.29 ± 0.08	2.20 – 2.55
Magnesium (mmol/L)	0.91 ± 0.06	0.87 ± 0.06	0.70 – 0.95
Total protein (g/L)	69.52 ± 2.46	71.71 ± 5.03	66 – 79
Albumin (g/L)	47.38 ± 2.15	48.68 ± 2.42	39 – 50
Globulin (g/L)	22.13 ± 2.04	23.04 ± 3.36	-
Total billirubin (μmol/L)	9.76 ± 3.01	11.49 ± 8.78	4 – 30
Unconjugated billirubin (μmol/L)	6.23 ± 2.12	7.85 ± 6.79	-
Conjugated billirubin (μmol/L)	3.53 ± 0.98	3.64 ± 2.04	0 – 8
Alanine phosphatase (ALP) (UI/L)	72.82 ± 26.99	84.40 ± 26.29	38 – 102
γ-gluthamyl transferase (GGT) (UI/L)	17.35 ± 4.22	22.89 ± 9.61	8 – 32
Alanine transferase (ALT) (UI/L)	19.40 ± 13.44	25.84 ± 13.50	6 – 32
Aspartate transferase (AST) (UI/L)	33.16 ± 32.37	26.50 ± 9.03	9 – 34
Lactate dehydrogenase (LD) (IU/L)	156.73 ± 44.24	147.03 ± 31.70	90 – 180
Cholesterol (mmol/L)	3.70 ± 0.48	4.81 ± 1.11	3.5 – 5.7
LDL-Cholesterol (mmol/L)	2.09 ± 0.60	3.23 ± 1.06	1 – 3.3
HDL-Cholesterol (mmol/L)	1.27 ± 0.25	1.07 ± 0.19	1 – 2.7
Triglyceride (mmol/L)	0.76 ± 0.33	1.17 ± 0.49	0.3 – 2.3
Glucose (mmol/L)	4.67 ± 0.36	5.05 ± 0.28	3.5 – 6.2
Insulin (ρmol/L)	144.12 ± 39.84	167.55 ± 70.03	-
Osmolarity (mmol/kg)	284.40 ± 2.53	283.09 ± 1.58	275 – 300
Total antioxidant status (mmol/L)	1.24 ± 0.07	1.24 ± 0.08	-

ϕNormal range as used by the Department of Chemical Pathology, UP, Pretoria
SD = Standard deviation
*** Significant difference between groups for each parameter (independent sample t-test, $p \leq 0.05$)**

ADDENDUM F

RESULTS OF SPEARMAN'S CORRELATION COEFFICIENTS

Table 1: **Spearman's correlation coefficient between the changes in serum variables at different times and the changes in the fibrin network structure characteristics over the same time of active males for the high GI meal intervention***

Variables	ΔT 4 – 5 (Fast - 1h post-meal)			ΔT5– 6(1h post-meal - max. exercise)			ΔT 6 – 7 (Max. exercise - recovery)		
	MLR	K_s	Comp	MLR	K_s	Comp	MLR	K_s	Comp
Haematocrit	-0.333 0.225	-0.290 0.294	-0.441 0.100	-0.514 0.060	**-0.567** **0.043**	-0.182 0.551	**-0.710** **0.004**	**-0.680** **0.010**	0.205 0.502
Albumin	-0.191 0.495	0.008 0.979	**-0.698** **0.004**	0.086 0.771	0.280 0.354	-0.088 0.775	0.464 0.095	0.412 0.162	0.137 0.656
Total protein	-0.287 0.300	0.204 0.465	-0.495 0.061	0.068 0.817	0.176 0.566	0.011 0.972	0.251 0.387	0.275 0.363	0.338 0.258
Triglyderide	-0.160 0.569	-0.088 0.755	0.022 0.939	-0.345 0.227	-0.060 0.845	0.445 0.128	0.073 0.805	-0.143 0.642	0.451 0.122
Glucose	0.494 0.061	0.258 0.354	0.261 0.347	-0.147 0.615	0.099 0.748	0.099 0.748	-0.011 0.970	-0.033 0.915	0.126 0.681
Insulin	0.236 0.398	0.389 0.152	0.368 0.177	-0.152 0.605	-0.165 0.590	0.066 0.831	-0.024 0.935	0.110 0.721	0.115 0.707
Fibrinogen	-0.354 0.196	0.209 0.454	0.084 0.766	-0.437 0.118	0.088 0.775	0.275 0.364	-0.108 0.714	-0.027 0.929	0.434 0.138
FVIIc	-0.450 0.092	-0.130 0.643	**-0.529** **0.043**	-0.068 0.817	-0.038 0.901	-0.225 0.459	0.077 0.794	-0.104 0.734	-0.297 0.325
Variables	**ΔT4 – 6 (Fast – max. exercise)**			**ΔT4 – 7(Fast - recovery)**			**ΔT5–7(1h post-meal - recovery)**		
	MLR	**K_s**	**Comp**	**MLR**	**K_s**	**Comp**	**MLR**	**K_s**	**Comp**
Haematocrit	**-0.618** **0.019**	-0.226 0.459	-0.039 0.899	-0.071 0.802	0.193 0.491	**-0.533** **0.041**	0.097 0.732	-0.108 0.702	0.009 0.974
Albumin	-0.248 0.392	0.231 0.448	0.104 0.734	-0.104 0.712	-0.312 0.258	0.143 0.610	0.007 0.980	-0.420 0.119	-0.394 0.147
Total protein	-0.336 0.240	0.159 0.603	0.121 0.694	0.018 0.950	-0.186 0.508	0.289 0.296	-0.029 0.919	-0.427 0.112	-0.397 0.143
Triglyceride	-0.358 0.208	0.027 0.929	0.214 0.482	-0.186 0.508	-0.314 0.254	-0.186 0.508	-0.311 0.260	-0.150 0.594	-0.111 0.694
Glucose	-0.112 0.703	0.198 0.517	0.468 0.107	0.016 0.955	0.309 0.262	0.013 0.965	-0.202 0.470	0.141 0.616	0.109 0.699
Insulin	-0.292 0.311	-0.527 0.064	-0.132 0.668	0.329 0.232	0.268 0.334	0.271 0.328	-0.168 0.550	.493 .062	0.127 0.652
Fibrinogen	-0.358 0.208	0.000 1.000	-0.374 0.209	-0.250 0.369	-0.075 0.791	-0.114 0.685	-0.118 0.676	0.132 0.639	-0.347 0.205
FVIIc	-0.305 0.288	-0.495 0.086	-0.495 0.086	0.082 0.771	0.107 0.704	0.271 0.328	0.089 0.752	0.068 0.810	-0.313 0.256

Table 2: Spearman's correlation coefficient between the changes in serum variables at different times and the changes in the fibrin network structure characteristics over the same time of active males for the low GI meal intervention*

Variables	ΔT 8 – 9 (Fast - 1h-post meal)			ΔT9–10(1h post-meal -max. exercise)			ΔT10– 11 (Max. exercise - recovery)		
	MLR	K_s	Comp	MLR	K_s	Comp	MLR	K_s	Comp
Haematocrit	-0.331 0.228	-0.429 0.110	0.057 0.841	0.203 0.469	0.217 0.436	0.080 0.777	0.029 0.919	0.110 0.697	0.287 0.300
Albumin	0.082 0.771	0.227 0.417	-0.090 0.751	0.357 0.191	-0.021 0.940	0.214 0.443	-0.404 0.136	**-0.571** **0.026**	-0.136 0.629
Total protein	0.181 0.519	0.179 0.524	-0.248 0.374	0.320 0.245	-0.166 0.554	0.209 0.454	-0.461 0.084	-0.511 0.052	0.070 0.805
Triglyderide	-0.183 0.514	-0.068 0.809	-0.255 0.359	0.424 0.116	-0.045 0.874	0.102 0.718	-0.075 0.791	-0.425 0.114	-0.021 0.940
Glucose	0.238 0.392	-0.462 0.083	-0.251 0.367	0.436 0.104	-0.229 0.413	0.200 0.475	-0.004 0.990	-0.204 0.467	0.263 0.344
Insulin	-0.111 0.694	-0.196 0.483	0.132 0.639	0.121 0.666	-0.146 0.603	**0.521** **0.046**	0.389 0.152	0.386 0.156	0.136 0.629
Fibrinogen	**-0.543** **0.037**	0.150 0.594	0.304 0.271	**-0.600** **0.018**	-0.089 0.752	0.086 0.761	**-0.583** **0.022**	-0.464 0.081	-0.086 0.761
FVIIc	0.104 0.712	0.036 0.899	0.178 0.527	0.050 0.860	0.139 0.621	0.464 0.081	-0.004 0.990	0.011 0.970	0.229 0.412
Variables	**ΔT8 – 10 (Fast – max. exercise)**			**ΔT8– 11(Fast - recovery)**			**ΔT9–11(1h post-meal - recovery)**		
	MLR	K_s	Comp	MLR	K_s	Comp	MLR	K_s	Comp
Haematocrit	0.291 0.293	-0.032 0.911	0.133 0.635	-0.177 0.527	-0.091 0.748	-0.123 0.663	-0.011 0.969	-0.024 0.934	0.326 0.235
Albumin	0.043 0.879	0.176 0.531	0.201 0.473	0.168 0.550	-0.143 0.612	0.250 0.369	-0.050 0.860	-0.221 0.428	-0.082 0.771
Total protein	-0.157 0.576	0.089 0.752	0.218 0.435	0.296 0.283	-0.125 0.657	0.296 0.283	-0.221 0.428	-0.386 0.156	-0.196 0.483
Triglyceride	**0.604** **0.017**	0.150 0.594	0.171 0.541	-0.204 0.467	-0.279 0.315	-0.193 0.491	0.007 0.980	**-0.539** **0.038**	-0.407 0.132
Glucose	0.239 0.390	-0.011 0.970	0.371 0.173	0.282 0.308	-0.507 0.054	-0.354 0.196	0.382 0.160	-0.364 0.182	-0.179 0.524
Insulin	0.061 0.830	0.375 0.168	0.125 0.657	0.025 0.930	0.000 1.000	0.021 0.940	0.236 0.398	0.189 0.499	-0.004 0.990
Fibrinogen	-0.404 0.136	-0.104 0.713	-0.300 0.277	**-0.546** **0.035**	-0.246 0.376	0.079 0.781	**-0.757** **0.001**	0.161 0.567	-0.007 0.980
FVIIc	-0.096 0.732	0.046 0.869	-0.254 0.362	-0.071 0.800	0.407 0.132	0.193 0.491	0.114 0.685	**0.514** **0.050**	-0.175 0.533

Table 3: Spearman's correlation coefficient between the changes in serum variables at different times and the changes in the fibrin network structure characteristics over the same time of sedentary males for the high GI meal intervention*

Variables	ΔT 4 – 5 (Fast - 1h post-meal)			ΔT5– 6(1h post-meal - max. exercise)			ΔT 6 – 7 (Max. exercise - recovery)		
	MLR	K_s	Comp	MLR	K_s	Comp	MLR	K_s	Comp
Haematocrit	0.393	-0.012	-0.055	-0.572	-0.618	-0.615	0.267	0.632	-0.076
	0.295	0.973	0.880	0.108	0.103	0.078	0.456	0.068	0.835
Albumin	-0.043	0.058	0.000	-0.100	-0.119	-0.250	-0.212	0.133	0.115
	0.912	0.874	1.000	0.798	0.779	0.516	0.556	0.732	0.751
Total protein	-0.146	0.158	0.021	-0.267	-0.048	-0.317	0.006	0.433	0.188
	0.708	0.664	0.955	0.488	0.911	0.406	0.987	0.244	0.603
Triglyderide	0.387	0.232	0.073	-0.233	**-0.810**	-0.583	0.406	0.467	-0.503
	0.304	0.519	0.841	0.546	**0.015**	0.099	0.244	0.205	0.138
Glucose	-0.192	0.374	0.534	-0.400	0.000	0.583	-0.370	-0.283	0.164
	0.620	0.287	0.112	0.286	1.000	0.099	0.293	0.460	0.651
Insulin	-0.017	0.212	0.491	-0.217	0.357	**0.783**	-0.588	-0.233	**0.685**
	0.966	0.556	0.150	0.576	0.385	**0.013**	0.074	0.546	**0.025**
Fibrinogen	-0.117	0.608	**0.711**	-0.500	-0.024	0.133	0.042	0.550	0.297
	0.764	0.062	**0.021**	0.170	0.955	0.732	0.907	0.125	0.405
FVIIc	**-0.700**	0.128	0.298	-0.467	0.167	0.417	0.176	0.383	0.370
	0.036	0.725	0.403	0.205	0.693	0.265	0.627	0.308	0.293
Variables	**ΔT4 – 6 (Fast – max. exercise)**			**ΔT4 – 7(Fast - recovery)**			**ΔT5–7(1h post-meal - recovery)**		
	MLR	**K_s**	**Comp**	**MLR**	**K_s**	**Comp**	**MLR**	**K_s**	**Comp**
Haematocrit	0.145	0.222	0.037	0.452	0.389	-0.098	0.324	-0.459	-0.107
	0.733	0.565	0.919	0.190	0.267	0.774	0.361	0.214	0.769
Albumin	0.143	0.067	0.309	-0.345	0.091	0.564	0.127	-0.433	-0.103
	0.736	0.865	0.385	0.328	0.803	0.071	0.726	0.244	0.777
Total protein	-0.119	-0.200	0.248	-0.358	-0.067	0.473	0.073	-0.433	-0.055
	0.779	0.606	0.489	0.310	0.855	0.142	0.841	0.244	0.881
Triglyceride	0.405	**0.733**	0.515	0.394	0.297	-0.364	-0.491	-0.233	-0.515
	0.320	**0.025**	0.128	0.260	0.405	0.272	0.150	0.546	0.128
Glucose	-0.595	0.317	0.248	-0.170	-0.249	0.082	0.285	**0.817**	0.539
	0.120	0.406	0.489	0.638	0.487	0.811	0.425	**0.007**	0.108
Insulin	-0.262	0.200	0.285	-0.370	-0.079	0.564	0.455	**0.767**	**0.673**
	0.531	0.606	0.425	0.293	0.829	0.071	0.187	**0.016**	**0.033**
Fibrinogen	-0.286	0.017	0.224	0.103	-0.030	-0.109	-0.455	-0.250	-0.576
	0.493	0.966	0.533	0.777	0.934	0.750	0.187	0.516	0.082
FVIIc	-0.119	-0.050	0.224	0.024	0.043	-0.100	-0.152	-0.433	-0.442
	0.779	0.898	0.533	0.947	0.907	0.769	0.676	0.244	0.200

Table 4: **Spearman's correlation coefficient between the changes in serum variables at different times and the changes in the fibrin network structure characteristics over the same time of sedentary males for the low GI meal intervention***

Variables	ΔT 8 – 9 (Fast - 1h post-meal)			ΔT9–10(1h post-meal -max. exercise)			ΔT10–11(Max. exercise - recovery)		
	MLR	K_s	Comp	MLR	K_s	Comp	MLR	K_s	Comp
Haematocrit	0.083 0.808	-0.491 0.125	-0.362 0.274	0.378 0.252	0.358 0.310	0.574 0.083	0.160 0.639	0.231 0.522	0.143 0.694
Albumin	0.124 0.715	-0.005 0.989	-0.192 0.572	-0.118 0.729	-0.345 0.328	0.030 0.934	-0.327 0.326	-0.407 0.243	-0.285 0.425
Total protein	0.115 0.736	-0.143 0.676	-0.168 0.621	0.027 0.937	-0.309 0.385	0.079 0.829	-0.455 0.170	-0.584 0.077	-0.442 0.200
Triglyderide	**0.645** **0.032**	0.245 0.467	0.583 0.060	**0.627** **0.039**	0.309 0.385	0.455 0.187	0.309 0.355	0.018 0.960	0.321 0.365
Glucose	-0.409 0.211	0.409 0.211	0.111 0.746	-0.036 0.915	0.224 0.533	-0.055 0.881	0.191 0.574	0.049 0.894	0.006 0.987
Insulin	-0.036 0.915	0.282 0.401	0.128 0.401	0.455 0.160	0.479 0.162	0.345 0.328	**0.855** **0.001**	0.565 0.089	**0.830** **0.003**
Fibrinogen	-0.519 0.102	0.287 0.392	0.183 0.591	-0.136 0.689	-0.309 0.385	-0.406 0.244	-0.155 0.650	-0.061 0.868	-0.479 0.162
FVIIc	0.006 0.987	-0.273 0.446	-0.383 0.275	-0.127 0.726	-0.317 0.406	0.233 0.546	0.527 0.096	0.401 0.250	0.40 0.214
Variables	**ΔT8 – 10 (Fast – max. exercise)**			**ΔT8 – 11(Fast - recovery)**			**ΔT9–11(1h post-meal - recovery)**		
	MLR	K_s	Comp	MLR	K_s	Comp	MLR	K_s	Comp
Haematocrit	0.405 0.216	0.030 0.934	0.097 0.789	0.266 0.429	-0.154 0.651	-0.215 0.526	-0.546 0.082	-0.344 0.300	-0.385 0.242
Albumin	-0.364 0.272	-0.139 0.701	-0.127 0.726	0.000 1.000	0.409 0.212	0.191 0.574	0.045 0.894	-0400 0.223	-0.364 0.272
Total protein	-0.400 0.223	-0.042 0.907	-0.018 0.960	-0.087 0.800	0.424 0.194	0.187 0.582	0.046 0.894	-0.282 0.400	-0.364 0.270
Triglyceride	0.291 0.385	0.236 0.511	0.067 0.855	0.145 0.670	0.300 0.370	0.000 1.000	0.169 0.620	-0.059 0.863	-0.050 0.884
Glucose	0.082 0.811	0.224 0.533	0.309 0.385	0.018 0.958	0.105 0.759	0.255 0.449	0.227 0.502	0.582 0.060	0.300 0.370
Insulin	**0.618** **0.043**	0.612 0.060	**0.636** **0.048**	0.091 0.790	-0.036 0.915	0.136 0.689	0.318 0.340	**0.727** **0.011**	0.536 0.089
Fibrinogen	-0.064 0.853	-0.067 0.855	-0.079 0.829	-0.336 0.316	0.373 0.259	0.182 0.593	**-0.827** **0.002**	-0.318 0.340	-0.209 0.537
FVIIc	0.518 0.102	0.018 0.960	-0.212 0.556	-0.036 0.915	-0.118 0.729	-0.491 0.125	-0.176 0.627	0.042 0.907	0.103 0.777

MLR	Mass–length ratio
K_s	Permeability
Comp	Compaction
FVIIc	Factor VII
Max. exercise	Maximal exercise
Recovery	30 minutes of recovery
GI =	Glycaemic index

T1	Fasting
T2	Maximal exercise after fasting
T3	30 minutes of recovery from maximal exercise after fasting
T4	Fasting before high GI meal
T5	1h post-high GI meal
T6	Maximal exercise after high GI meal
T7	30 minutes of recovery from maximal exercise after high GI meal
T8	Fasting before low GI meal
T9	1h post-low GI meal
T10	Maximal exercise after low GI meal
T11	30 minutes of recovery from maximal exercise after low GI meal